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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
BPW	Buffered peptone water
CDC	Centers for Disease Control and Prevention
CSPI	Centre for Science for Public Interest
CIAMD	Centre of International Agricultural Marketing and Development
CSIR	Council for Scientific and Industrial Research
DFPT	Deciduous Fruit Producers' Trust
DAFF	Department of Agriculture, Forestry and Fisheries
DWAF	Department of Water Affairs, Agriculture and Forestry
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agricultural Organisation
GAP	Good agricultural practices
HACCP	Hazard analysis and critical control points
HSMS	Horticultural Safety Management System
MPN	Most probable number
NB	Nutrient broth
PPECB	Perishable Products Export Control Board
PCR	Polymerase chain reaction
SEM	Scanning electron microscopy
TSB	Tryptone soy broth
TB	Tryptose broth
UK	United Kingdom
FDA	United States Food and Drug Administration
USA	United States of America
WHO	World Health Organisation

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Chapter 1

General Introduction

Consumption of fresh produce has increased over the past ten years and it is expected that this trend will continue over the next few years due to the associated positive health attributes (Dillard and German, 2000). Human nutrition research shows that fruit and vegetables contribute to a well-balanced diet and general good health (Meng and Doyle, 2002). With the greater demand for fresh produce worldwide, an increase in productivity is required and improved global distribution networks (Jacxsens *et al.*, 2010). This requires more complex and longer food chains, increasing the risk of product contamination. With an increase in demand for fresh produce supplied throughout the year there has been growing trend to source from developing countries which are generally considered more likely to supply contaminated produce and represent a higher risk (Dillard and German, 2000).

Contamination of food with human pathogens has been investigated extensively within the past century (Bean and Griffin, 1990; Garcia-Villanova Ruiz *et al.*, 1987; Lindqvist *et al.*, 2000; Notermans and Borgdorff, 1997). Foodborne outbreaks are sporadic and have been reported to have caused at least 5 000 deaths in the United States of America (USA) annually (Gerner-Smidt, 2006). The most important of these pathogens that have been found to be associated with fresh produce includes some of the following: *Escherichia coli* which have been detected from surfaces of plums (Abdelnoor *et al.*, 1983), strawberries [Centers for Disease Control and Prevention (CDC), 2006] and table grapes [Centre for Science for Public Interest (CSPI), 2008], *Salmonella* spp. which have been detected on mango (Sivapalasingam *et al.*, 2003), cantaloupe (CSPI, 2008), plums (CDC, 2006), strawberries (CDC, 2006; CSPI, 2008) and table grapes (CSPI, 2008) and *Staphylococcus aureus* was detected on lemons (CSPI, 2008) and cantaloupe (CSPI, 2008). The presence of these foodborne pathogens does not necessarily lead to an outbreak of foodborne disease. Furthermore, the presence of these pathogens at harvest or further down the supply chain is not necessary indicative of the risk at the final point of consumption. These pathogens have also been detected from the processed products for example *E. coli*, *Salmonella* spp. and *S. aureus* have been reported to be found in apple juice (Besser *et al.,* 1993, CDC, 1975), orange juice (CDC, 2005) and banana pudding (CDC, 2004), respectively.

Food contamination can happen at any stage between production and packing or processing and can be due to poor quality water, inadequate personal hygiene or facility sanitation. A lack of knowledge of potential contamination points can lead to an increased risk of product contamination while moving through the chain (De Roever, 1998). Water quality changes seasonally and is often influenced by upstream activities (De Roever, 1998). Since water comes into direct contact with fruit when the trees are irrigated or when pesticides are applied and later during postharvest washing and pre-cooling conditions, food safety assurance may be compromised when the water is contaminated. Consumers are also now more aware of the risks associated with contaminated food and demand a safer food supply. Therefore food safety assurance has become an important compliance criterion to gain market access as well as to ensure consumer confidence.

Increased global trade has shifted the focus towards food safety assurance. This shift is due to the perceived increased risks, sheer volumes of fresh produce being traded and the complex food distribution networks between and within countries (Hall *et al.*, 2002). A further shift towards the implementation of required national regulation and voluntary international food safety standards has recently been seen. This has forced industries to determine the potential risk associated with the consumption of fresh produce. In order for risks to be quantified, scientific evidence needs to be available. These data then allow scientifically informed decisions to be made based on microbiological criteria in order to support and improve food safety management systems (Jacxsens *et al.*, 2010). Fruit exported from developing countries are thought to pose a greater threat. This perceived threat is due to perceived unhygienic production and packing practices. The use of potentially contaminated water for irrigation, pesticide spraying and washing in packhouses also adds to the perceived threat. This resulted in the implementation of voluntary and regulatory good agricultural practices and food safety standards for primary producers. Therefore, to improve safety of produce, it is essential that all role players address specific food management systems according to their needs as the product moves from the farm to the table (Ropkins and Beck, 2000). Food safety issues must therefore focus on all aspects of production and packing and on all stages in the farm-to-table food chain. The assessment of risk needs to focus on detection methods that allow the accurate detection of pathogens from crop and contact surfaces, detection of those pathogens and simulation modelling to determine if pathogens are able to survive (Jacxsens *et al*., 2010).

Europe is a major importer of fresh produce and retailers in the European Union (EU) therefore insist on food safety assurance (Dorling *et al*., 2008 as cited by Jacxsens *et al.*, 2010). With the global move towards food safety assurance systems (Jacxsens *et al.*, 2010),

the South African agricultural industry finds itself in a predicament of either conforming to world standards or be excluded from international trade. The South African fresh produce export market is especially important because it is a source of foreign revenue, food security and job creation. The South African fruit export industry is of significant importance due to high levels of foreign revenue generated. Fruit export accounts for 50% of the country's agricultural production [Perishable Products Export Control Board (PPECB), 2006]. As with the other major fruit types the South African stone fruit industry is export driven with plums being delivered mainly to the European market (55.8%) and peaches mainly to the Middle East and Mediterranean (36.97%) (in the 2008/2009 season) (PPECB, 2010). Although the stone fruit industry is a small player in terms of fruit exports it remains a strong competitive industry in the international market due to a large number of successful cultivars (South African Fruit Farms, 2007).

Food safety assurance and sanitary and phytosanitary issues are the most important aspects that threaten continued export of South African fresh produce to first world countries. South Africa has a long and proud history of exporting quality produce to mainly European markets and more recently to alternative markets such as the USA, Middle East, and Asia. With globalisation, new threats and opportunities that require a different focus for South African fruit growers and exporters have emerged. International concern over the indiscriminate use of pesticides and the increased occurrence of foodborne illnesses, as well as a range of new emerging diseases, has resulted in retailers and market forces demanding safe produce within a framework of an accountable food safety system approach (Jacxsens *et al*., 2010). Countries that cannot adhere to these basic food safety requirements will be excluded from the international trade arena and denied market access in the global village. South African farmers have implemented food safety management systems to remain in the international trade arena. In addition, national requirements and legislation will have to ensure protection for the consumer in both local and export products.

Food safety standards and systems that target zero microbial contamination have been developed specifically for the food processing industries. The same principles have only recently been adopted for the fresh produce sector. However, since microbiological analysis of food is time consuming, the International Commission on Microbiological Specification for Foods stated in 1986 that "good agricultural practices (GAP) and acceptable hygienic farming practices are more important than microbiological testing of food samples before selling" (Food Science Australia, 2000). However, compliance with standard microbiological criteria remains the key to the development of an effective hazard analysis and critical control points (HACCP) system for proper risk control (Buchanan, 1995).

A holistic view of the production and processing environment, as demonstrated in Figure 1, is required to ensure that a complete assessment is conducted. The complete view includes the pre- and post-harvest environment, the natural, resident and transient microflora, the host attributes as well as the contribution of human activity to the safety of the fruit. Human activity is the only completely controllable area within the holistic framework.

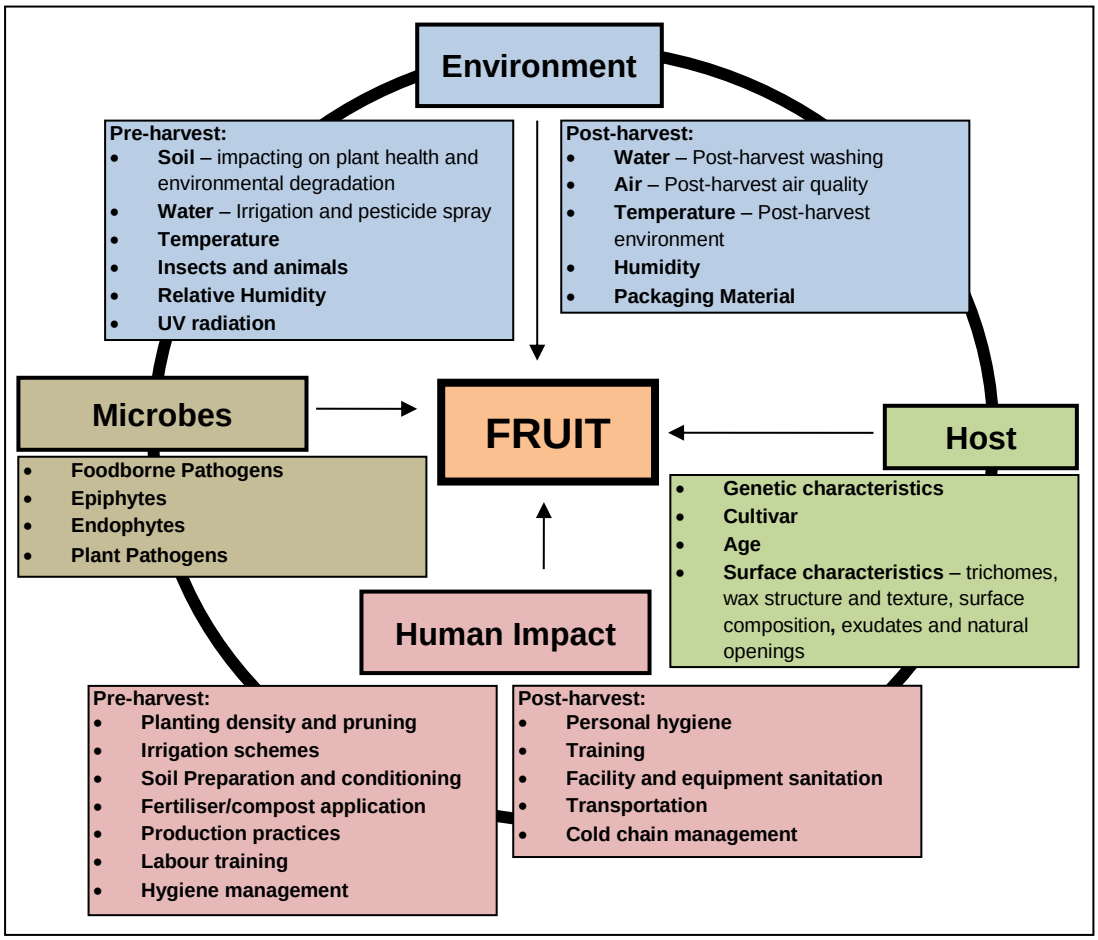

Figure 1.1: Important parameters that impact on the safety and quality of fruit.

In order to test the hypothesis that water and contact surfaces do not pose a risk on the safety of stone fruit a number of aspects were tested. An accurate, robust and reliable detection technology needed to be developed to the target organisms in this study (*Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus*) which is outlined in Chapter three. The multiplex PCR was developed to replace the laborious and time consuming traditional methods using selective cultural media, followed by various possible identification technologies that have been proven to be less sensitive or specific than a polymerase chain reaction (Aznar and Solis, 2006; Odumeru et al., 1999). Once an accurate and effective detection technology was developed and verified it was used to test for the presence of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* on fruit, in water

and on contact surfaces within the peach and plum production environments, which is outlined in Chapter four and five. The presence of these pathogens has become an important issue for industry to address, due to increased global pressure for food safety assurance and in the light of the increasing water contamination problem in South Africa. It was also important to determine the growth dynamics and ability of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* to attach and colonise the surfaces of stone fruit and to what extent this was possible and to determine whether these organisms were able to survive a simulated export chain, which is reported on in Chapters six and seven. The detection and attachment, colonisation and survival studies were required to conduct a risk assessment of the peach and plum production environment. A semi-quantitative risk assessment was conducted and is outlined in Chapter eight and finally an evaluation of all farms' safety management systems were conducted as outlined in Chapter nine. All these aspects give a full picture of the primary production fruit safety of peach and plum production, allowing us to determine areas where the safety management system can be improved.

The exploration of this hypothesis has resulted in a developed basic food safety framework for the South African stone fruit industry by providing sound scientific evidence of the level of risk. The risk-based approach provides scientific evidence for actual hazards and allows characterization of true risks. This project therefore aims to address principal elements known to give rise to microbial food safety concerns, which can be summarised as:

- Prevention of microbial contamination of fresh fruit, which is favoured over reliance on corrective actions once contamination has occurred.
- To minimize microbial food safety hazards associated with fresh stone fruit, by using good agricultural and management practices and food safety management systems in areas where control can be exerted.
- Whenever water comes in contact with fresh stone fruit, its source and quality dictates the potential for microbial contamination. This, determines the potential of microbial cross-contamination from polluted agricultural water used to irrigate tree crops.
- Wherever fruit comes into contact with a tainted surface it could represent a potential source of contamination. Attachment, colonisation and survival of foodborne pathogens to and on fruit surfaces during the supply chain can increase the risk of a potential contamination by allowing the proliferation of these organisms, therefore increasing the load of these organisms on the produce.

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Chapter 2

Stone Fruit Safety: Means and prevention of contamination and detection of foodborne pathogens

Abstract

Contamination of food with human pathogens has been investigated extensively within the past century. Contamination of food, and more recently of fresh produce, with foodborne pathogens is of concern due to an increase in immunocompromised individuals, global procurement patterns and movement of food. Global distribution of food products requires longer food chains, increasing the risk of contamination due to increased handling and inadequate hygiene. A lack of knowledge of the risks associated with poor personal hygiene and inadequate facility sanitation, can lead to an increased risk of contaminating the product while being moved through the chain. Water availability, quality and management are a global concern as in South Africa. Water quality changes seasonally depend on upstream activities that could affect fruit safety, since water comes into contact with fruit when pesticides are applied, through irrigation water, as well as washing and pre-cooling systems. Consumers are now more aware of the possibility of produce contamination and therefore food safety assurance has become important to ensure consumer confidence.

1. Introduction

Fruit are an important export product of South Africa with 1882.47 million dollars earned during the 2007 season [Food and Agricultural Organisation (FAO), 2010]. Deciduous stone fruit are cultivated in South Africa mainly in the Eastern and Western Cape on 60 653 ha representing plum orchards and 10 000 ha representing peach orchards during the 2008/2009 season. Due to the large amount of fresh produce exported from South African shores, contamination of the fruit is an important consideration (Bean and Griffin, 1990; Garcia-Villanova Ruiz *et al.*, 1987; Lindqvist *et al.*, 2000; Notermans and Borgdorff, 1997). Fresh produce can become contaminated during the various stages of production, packing, distribution or transportation through the cold chain. Product contamination could potentially lead to further proliferation of the pathogen present if the cold chain, locally or globally, is not managed correctly. The global distribution of food products requires more extensive food chains, increasing the risk of contamination due to increased handling and inadequate hygiene. This risk may be increased further due to a lack of knowledge linked with poor personal hygiene and inadequate facility sanitation (De Roever, 1998). Foodborne infections associated with food and more recently fresh produce (Todd, 1997), cause large threats to public health and have a major economic impact within a country. Public health threats are further increased due to a growing number of immunocompromised individuals, global procurement patterns and movement of fresh produce. The aim of this review is to outline all facets involved in growing stone fruit and highlights potential contamination points of fresh produce. The review also covers previous reports of disease outbreaks associated with fresh produce and the early detection and prevention of contamination and ultimately illness due to consumption of contaminated produce.

2. Stone Fruit

Deciduous stone fruit bear fleshy indehiscent fruit with a single seed. Peaches are soft juicy fruit with yellow flesh and a red-tinted yellow skin and a deeply sculptured stone containing a single seed. Peaches are native to China, but were named *Prunus persica* L. Batsch because it was thought to have originated in Persia (now Iran). However, it was later discovered that trees were taken to Persia in 2000 B.C. (Anonymous, 2007a). The historical peach cultivation areas are China, Japan, Turkey and Mediterranean countries. In 2009, 1.7 million hectares of land were under peach orchards worldwide with 18.6 million tonnes of peaches produced in the area. The world's largest producer of peaches in 2009 was China, with 802 686 ha of land under peach cultivation (FAO, 2010). Plums are smooth-skinned, fleshy fruit containing a stone that encloses a single seed. The Japanese Plum (*Prunus*

salicina) dates back to 479 B.C. and originated in China (Anonymous, 2007b). The European Plum (*Prunus domesticus*) originated near the Caspian Sea and was introduced into Northern America by pilgrims. China was the largest producer of plums in 2009, with 1 663 115 ha of land used for plum production, with 5.4 million tonnes produced (FAO, 2010).

It is not known exactly when stone fruit entered into South Africa but the export of the first consignment of deciduous fruit took place in 1892 by Percy Molteno from the Cape to the United Kingdom (UK) [Hurndall: Deciduous Fruit Producers' Trust (DFPT), 2005]. Following the beginning of deciduous fruit export, Harry Pickstone imported deciduous fruit trees (50 000) into South Africa from California, which lead to large areas of Constantia, Stellenbosch and the Hex River Valley being planted by 1894. With increased plantings the export of deciduous fruit increased from 22 000 fruit punnets exported in 1903 to 4.6 million punnets in 1938 increasing further to 7.9 million fruit punnets in 1958. Twenty years later, South Africa was thus exporting to 34 countries on four continents (Hurndall: DFPT, 2005).

In 2009, South Africa had 6500 ha of land under peach orchards and 10 000 ha plums produced. A number of areas produce stone fruit (Figure 2.1), with the largest being Eastern and Western Cape. In 2008, the Klein Karoo and Berg River produced 23% and 22% respectively of the 4 081 ha of plums cultivated in South Africa (DFPT, 2009). In the Ceres area 39% plums and 17% peaches are produced being the largest peach cultivation area of South Africa with total hectares being 8 490 ha (desert and cling peaches) (DFPT, 2009).

Plums and peaches produced in South Africa go into three possible avenues, export sale being the largest, followed by local sale and then processing. Currently the South African stone fruit industry is export driven with 43 742 tonnes of plums and 8 445 tonnes of peaches exported during 2007 (FAO, 2010). The 2007/2008 statistics showed that plums were mainly exported to Europe (55.8%) and peaches mainly to the Middle East and the Mediterranean (36.97%) (PPECB, 2010). Statistics from 2004 revealed that 12 239 tonnes of plums and 35 576 tonnes of peaches went into the local market, 47 085 tonnes of plums and 7 740 tonnes of peaches went into the export market and 2 545 tonnes of plums and 178 800 tonnes of peaches were processed into canned fruit, jams, dried fruit and juices [Centre of International Agricultural Marketing and Development (CIAMD), 2004 as cited by Hurndall: DFPT, 2005].

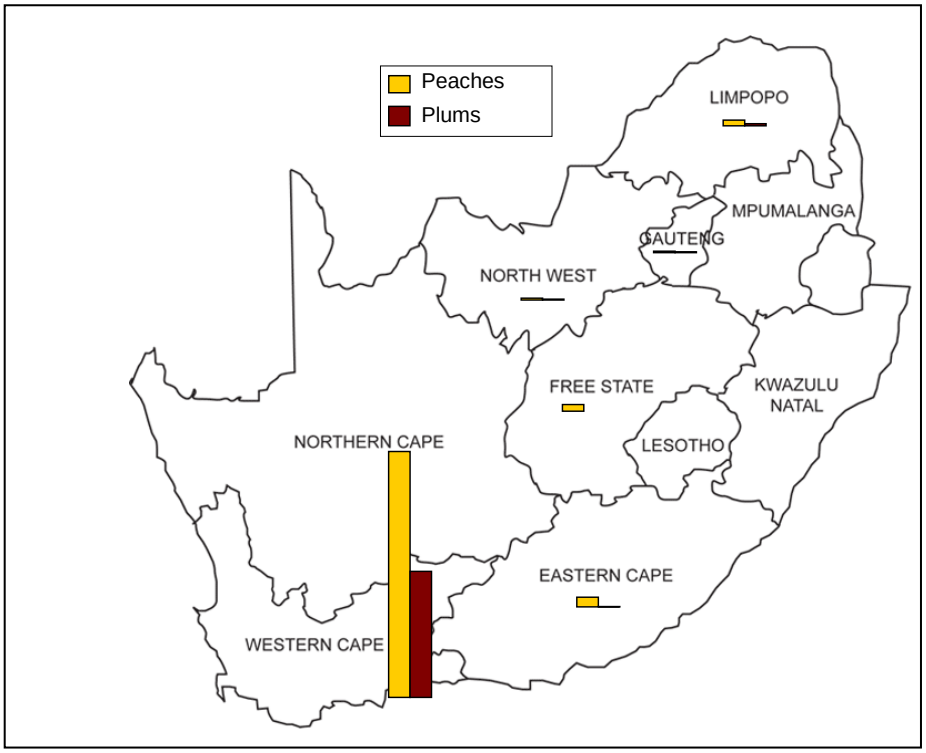

Figure 2.1: Main stone fruit producing areas of South Africa (DFPT, 2009).

The most economically important dessert peach cultivars in the 2007/2008 season were Transvalia with 373 373 cartons passed for export (DFPT, 2009). Transvalia is an early seasonal cultivar, harvested in mid-November (Hurndall: DFPT, 2005). The next most economically important peach, San Pedro, had 126 396 cartons passed for export in 2007/2008 season (DFPT, 2009). San Pedro is harvested in early November. In 2009/2010 season 120 665 metric tons of canning peaches were processed, mainly used for canning (82%) or puree (Canning Fruit Producers' Association, 2010) The largest production of canning peaches in South Africa in the 2007/2008 season was a yellow cling stone peach Keisie (1 419 hectares and 55 704 cartons released for export) (Hurndall: DFPT, 2005; DFPT, 2009). Kakamas, also a yellow skinned cling stone peach harvested in mid-February produced under 1 273 hectares of land with an export of 21 563 cartons is the second most important canning peach (Hurndall: DFPT, 2005; DFPT, 2009). The plum cultivar that was exported most during the 2007/2008 was Laetitia with 2.2 million cartons passed for export. Laetitia is produced only from 648 hectares of orchard land and is harvested in late January (Hurndall: DFPT, 2005; DFPT, 2009). The second most exported plum is a yellow variety called Songold. Songold's 1.64 million cartons were produced from 613 hectares of orchard land in 2007/2008 season. Songold is harvested in early February.

Trees are generally planted during the late winter or in early spring as a single block or a mixed cultivar block (two cultivars alternating in two rows (Hurndall: DFPT, 2005) with roots

remaining moist prior to planting. Trees are planted manually, except for some larger farms which use mechanical planting machines. Stone fruit are planted at a density of an average 1 250 trees per hectare but often the numbers are doubled. Three years after planting the first plums can be harvested and following two years for peaches, with the full production being reached after five years. Plum and peach trees can bear fruit and be commercially profitable for up to approximately 15 years.

Fruit must be harvested at the correct maturity and under the optimal conditions (SA Fruit Farms, 2010). Harvesting is conducted by hand using the best methodology/ harvesting aids and equipment. Fruit harvesting and packing requires extensive exposure to handling introducing a possible food safety risk if not done within an effective personal hygiene framework and regular training programme. Fruit should be harvested early in the morning when the temperature is 25^{o}C. When in the field fruit need to be kept in cool shaded areas and the field heat needs to be removed within three hours of harvest (Figure 2.2). The temperature of the fruit within the field could allow any potentially present organisms to survive and proliferate.

After harvest, fruit are transported within a half an hour to packhouse where the field heat is removed in a pre-cooling facility at a recommended 12^{o}C (Figure 2.2, 2.3 and 2.4) for three hours (SA Fruit Farms, 2010). Fruit are then moved into the packhouse where they are pre-sorted into fruit for the local or export market (Figure 2.2). Fruit are then sorted and graded according to set size specifications. During sorting, grading and packaging fruit come into direct contact with surfaces and hands, therefore potentially posing a food safety risk due to potential hazards on hands or contact surfaces in the facility (Figure 2.2). Fruit are then moved to the cold store (±0.5^{o}C) for storage until transportation to the specific market, the time of storage is dependent on the market and demand for fruit (Figure 2.2). Plums and peaches are exported according to recommendations provided by PPECB in order to guarantee the quality of the fruit. Peaches are exported at the recommended holding temperature for stone fruit, i.e. -0.5^{o}C for the full voyage from the exporting country to the importing country (Figure 2.2 and Figure 2.3), whereas plums are exported at -0.5^{o}C for 2 days with a subsequent ripening period where the temperature is increased to 7.5^{o}C for 5-10 days, after which the temperature is again lowered to -0.5^{o}C (Figure 2.2 and 2.4). Breaks in the cold chain management pose a food safety risk to fruit. The recommended temperatures are sometimes not adhered to due to logistical problems or ignorance and could potentially lead to a safety or quality breakdown, as indicated in Figure 2.3 and Figure 2.4 as red peaks. A number of observations have been made over the past few years which are a food safety concern. When fruit are transported to the harbour, it has been observed that transport is not

done in an adequately cooled truck and therefore fruit temperature increases. In addition breaks in shipment temperatures and during retail sale have been observed. The temperature of the fruit has been observed to increase prior to and after shipment due to delays in filling/emptying of containers or because the container was not adequately cooled. It has also been observed that during distribution to retailers the recommended temperatures are not adhered to due to logistical problems. Following purchasing the produce, consumers often do not refrigerate the product and therefore the fruit are not stored at the correct temperatures.

3. Threats to the South African Fruit Export Industry

South Africa has a long and proud history of exporting quality produce to mainly European markets. Through research and technology transfer, the South African stone fruit industry has been able to maintain their competitive edge and increase the market share. With globalisation, new threats and opportunities that require a different focus for South African fruit growers and exporters have emerged. The South African government established a Food Safety and Quality Assurance Directorate that falls under the National Department of Agriculture, Forestry and Fisheries. The Directorate controls the Standards Regarding Food Safety and Food Hygiene of Regulated Agricultural Food Products of Plant Origin destined for Export [as stipulated under 4(3)(a)(ii) of the Agricultural Products Standards Act 119 of 1990, and promulgated in notice R707 of 13 May 2005]. International concern over the indiscriminate use of pesticides and the increased occurrence of foodborne illnesses, as well as a range of new emerging diseases, has resulted in retailers and market forces demanding safe produce within a framework of the accountable systems approach. Global GAP and other voluntary standards have been established to ensure that produce being exported is of good quality and safety standards. Countries that cannot adhere to these basic food safety requirements will be excluded from the international arena and denied market access in the global village. For the South African economy to continue its growth and remain internationally competitive the country will have to adapt to these new requirements and implement international standards in food safety. In addition, national requirements and legislation will have to ensure protection for the consumer in both local and export products.

Countries can still set their own safety standards, but they must be based upon sound scientific evidence. The use of accepted international standards are encouraged and provide a much greater potential and safety assurance framework for fresh produce, particularly in developing countries. For safety, the main purpose is to ensure that products are not

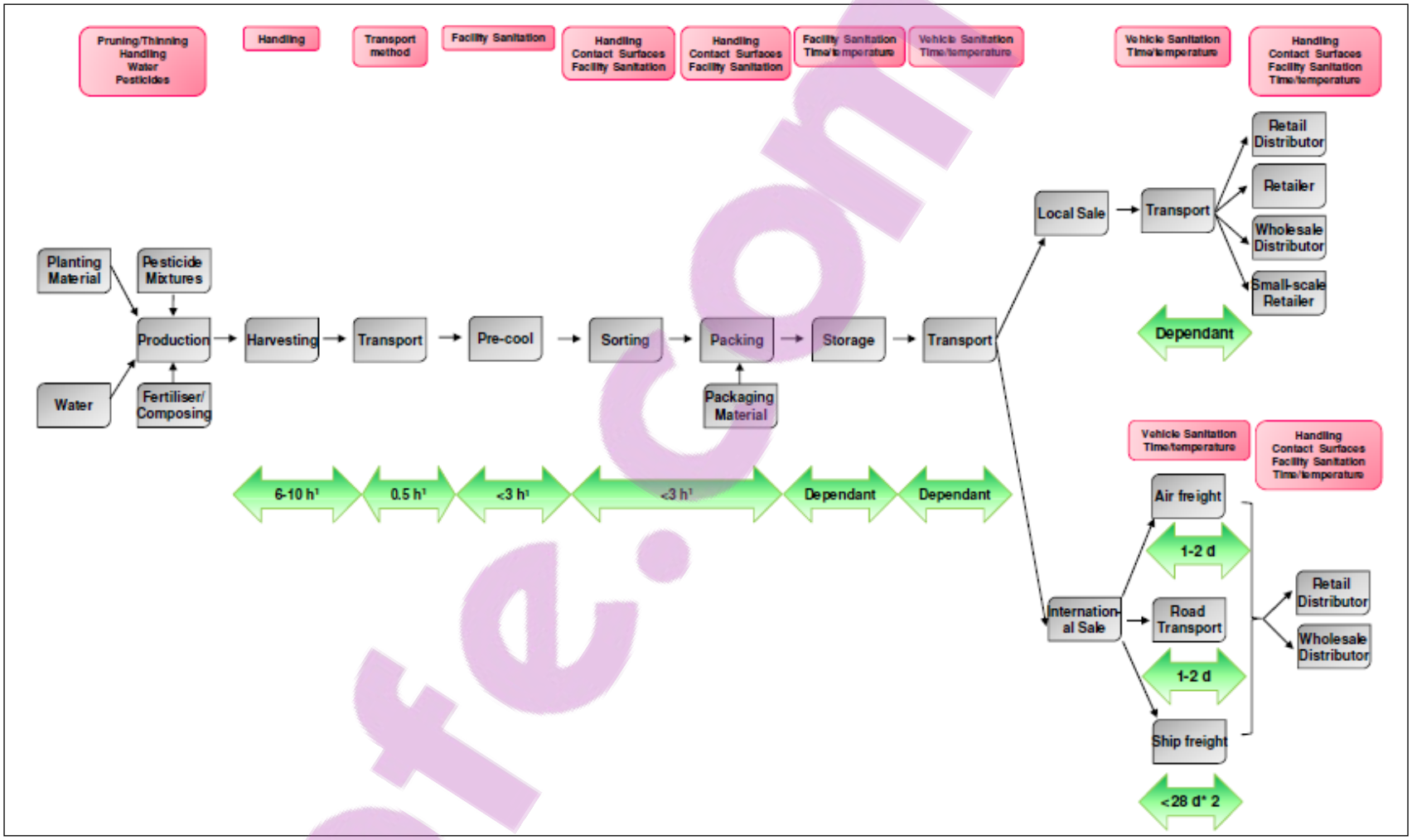

Figure 2.2: Stone fruit supply chain with the associated risks and recommended time intervals

Note: The risks are indicated in red and the time intervals in green

1: SA Fruit Farms, 2010; 2: Perishable Products Export Control Board, 2010

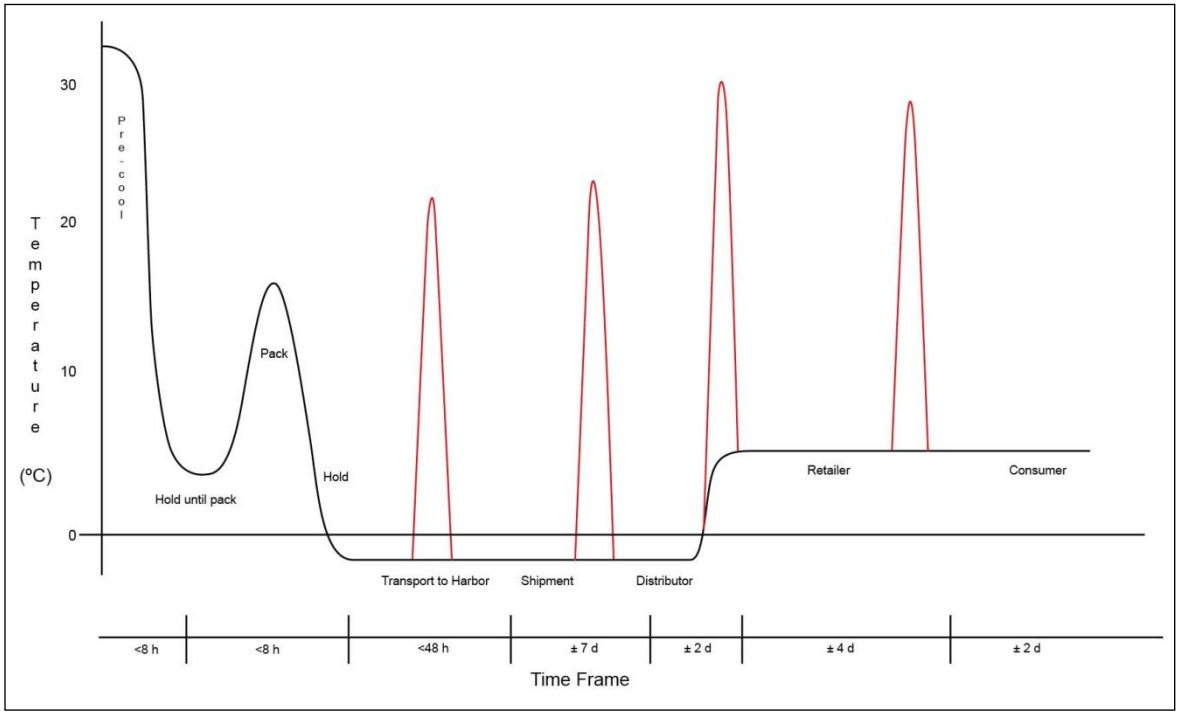

Figure 2.3: Temperature management in the peach export chain.

Note: Red peaks represent possible breaks in the cold chain temperature management.

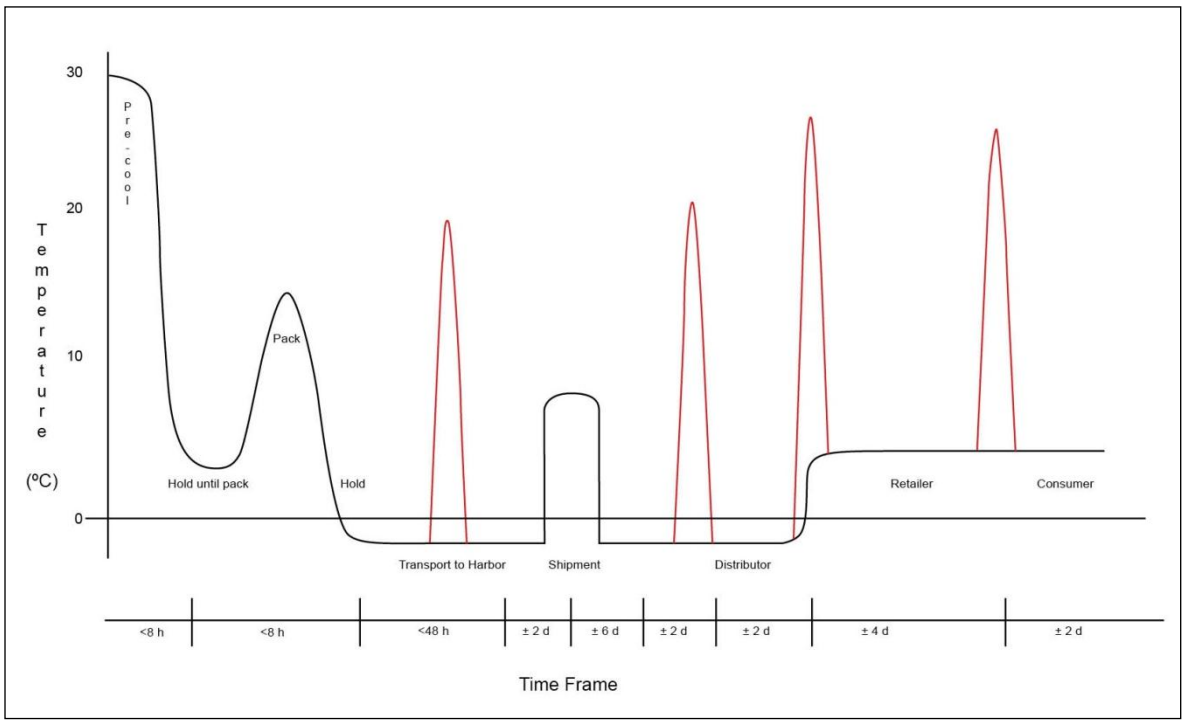

Figure 2.4: Temperature management in the plum export chain.

Note: Red peaks represent possible breaks in the cold chain temperature management.

adulterated nor carry dangerous contaminants such as undesirable microorganisms, pesticides or potential toxic components. The measurement and evaluation of safety standards can be complicated. Of particular concern to first world countries are the potential increase in risks associated with poorly controlled products and subsequent food safety risks of contaminated fresh produce. This is of importance when considering the spread of infectious diseases, foodborne pathogens and the newly emerging and resistant groups of pathogens.

4. Foodborne Pathogen Contamination within the Fresh Produce Cold Chain

Contamination can occur at any point through the cold chain, during production, processing or consumer contamination. The routes of contamination during production include manure, irrigation water, sewage and lack of field sanitation, during processing and retail include the lack of personal hygiene by handlers and poor facility sanitation, after retail includes poor handling and poor storage conditions (Brackett, 1999; James, 2006; Rajkowski and Baldwin, 2003).

4.1. Production sources

Events occurring prior to and during planting, as well as during production, can have a major influence on the safety of the produce (Brackett, 1999; James, 2006). All raw materials and facets of production could potentially lead to contamination. Production facets like the handling of water, pesticide mixtures and soil as raw materials and the handling of produce could ultimately lead to contamination (Brackett, 1999; De Roever, 1998; James, 2006). Irrigation, farming (fresh produce and animal) and sanitary practices, and personal hygiene of workers could potentially contribute to contamination of produce during growing, harvesting and packing if water of poor quality is used or if poor hygiene practices are followed (Brackett, 1999; Doyle, 2000; James, 2006).

Animal farming could potentially lead to a food safety risk of fresh produce when the field used for planting is contaminated with foodborne pathogens. Crops planted adjacent to animal-rearing operations have a high risk of contamination (James, 2006). A field previously used for grazing has a higher risk of contamination (Tauxe *et al.*, 1997) due to an organism's ability to survive in the soil for months to years (Brackett, 1999). *Escherichia coli* O157:H7 has been shown to survive in the soil for more than six months (Islam *et al.*, 2005). Water from rain runoff and rivers could flood over animal fields therefore increasing the possibility of faecal contamination due to the spreading of manure to fresh produce

production areas. In 1996, cattle grazing on an adjacent field were reported to be responsible for a multistate outbreak of *E. coli* O157:H7 in the USA [United States Food and Drug Administration (FDA), 1999; Hillborn *et al.*, 1999].

Good quality water is becoming a scarce commodity (Kirby *et al.*, 2003). Water quality and the supply source impacts on the safety of fresh produce. Irrigation water is usually sourced from ground water (an aquifer beneath the earth's surface), surface water (lakes, ponds or rivers) or wastewater (human sewage) (Steele and Odumeru, 2004). The most common source of agricultural water is surface water. The quality of surface water is difficult to control due to contamination by livestock, human activity and industrial processes (Kirby *et al.*, 2003; Steele and Odumeru, 2004). Contaminated water used could cause asymptomatic infection of the farm's livestock, thereby increasing the risk of produce contamination. Faecal contamination of water was decreased by 94% when a fence was constructed along the banks of a river to restrict access of livestock to the water (Hagedorn *et al.,* 1999).

Water quality is an important aspect when dealing with pesticide application or overhead irrigation. In both cases the liquid comes into direct contact with the fruit and potentially allows enough contact time for the attachment of the organisms. Guan *et al.* (2001) and Coghan (2000) independently stated that pesticides were stable environments for *Salmonella*, *E. coli* and *Listeria monocytogenes* and could therefore be a source of contamination (Ng *et al.*, 2005). Contamination of pesticides can occur in three different ways, namely (a) contaminated pesticide bought from the manufacturer or pesticides that are mishandled during distribution, storage and transportation, allowing contamination, (b) from the water used to dilute or reconstitute the pesticide and/or (c) the time between reconstitution and application could allow for the proliferation of contaminating organisms (Ng *et al.*, 2005). Ng *et al.* (2005) tested ten different products of which 10% allowed survival and 10% growth of *E. coli* O157:H7, 20% allowed the survival and 20% growth of *Salmonella* Typhimurium and 50% killed *L. monocytogenes* after only 12 hours. Not all products kill the pathogens of importance for food safety, demonstrating the importance of using an uncontaminated water source to reconstitute the product, in order to prevent contamination and possible proliferation.

Contamination of soil and planting material can occur via the use of improperly composted manure or sewage that is used as a fertiliser. Often soil is contaminated with faecally transmitted pathogens like different strains of *E. coli* (De Roever, 1998). The primary reservoir of *E. coli* O157:H7, the most important and virulent of strains, is cattle. *Escherichia coli* O157:H7 has been shown to persist in the rumen and colon of cattle, continuously

contaminating the faeces (Faith *et al.*, 1996; Islam *et al.*, 2005). If composting is carried out correctly then it will kill all pathogens in the heap due to the heat generated during composting. However, due to the variability of environmental conditions homogeneous conditions are not always created throughout the heap, allowing the survival of pathogens (Islam *et al.*, 2005). Millner (2003) found a 99.999% kill rate for *E. coli* O157:H7 and *Salmonella* sp. in a compost heap at 55^{o}C left for three consecutive days in an aerated heap or two weeks in a heap at 55^{o}C with five turnings. A microbial profile study done by Doyle (2000) on organic versus conventional produce, illustrated organisms were found more frequently and in higher numbers on the organic produce.

Animals, including birds and insects, play a role in the contamination of fruit (De Roever, 1998; James, 2006; Wallace *et al.*, 1997). Both birds and insects can travel substantial distances allowing the carriage of bacteria from one area to another. Animals (domestic and wild) could contaminate fruit, soil or water, leading to eventual contamination of fresh produce. Wallace *et al.* (1997) found that *Escherichia coli* O157:H7 was present in a small number (13; n= 200) of wild birds, mainly gulls. Wallace *et al*. (1997) however expressed concern for the potential of these birds to further infect cattle that come into contact with the contaminated faeces.

The hygiene practices of farm workers during land preparation, planting, weeding, pruning and harvesting influence the safety of the fruit (De Roever, 1998; James, 2006). Hands are an important vehicle in the faecal-oral transmission route of diarrhoeal disease, especially the hands of mothers and other caregivers (Saadé *et al.*, 2001). Many of these infections are preventable by good hand hygiene practice. One remarkable finding of a number of research studies is that improved hand hygiene has significant effect on preventing diarrhoeal disease, both in developing and developed countries. However, hand hygiene is not widely practiced.

Contaminated water used for drinking and hand washing can be a source of contamination of fruit within the farm environment, with contaminating organisms being transferred to the fruit. In a South African study of the effect of water quality on the outcomes of hand hygiene, Venter and September (2006) found varied results of the effect of water quality on hand hygiene. None of the hand hygiene procedures using highly polluted water resulted in the reduction of bacterial load on the hands. Also, no significant difference in bacterial loads was noticed between clean or moderately contaminated water used on hands. Venter and September (2006) concluded that hands with high bacterial load can be washed with water, even moderately contaminated water, to reduce microflora on the hands. Thus, although not

ideal, hand hygiene can still have a positive effect of reducing water-related diseases despite the quality of water used in the procedure.

In the 2006/2007 and 2007/2008 spot checks of completed project it was found that only 39% and 46% of the toilets, respectively, had a hand washing device (Duncker, *et al.*, 2007; Duncker, *et al.*, 2008). Similar findings were found during a study be the Council for Scientific and Industrial Research (CSIR) (2004). Such low numbers suggest that hand washing is not common practice within the homes of South Africans. Proper sanitary facilities with potable water and proper waste management need to therefore be provided and enforced to ensure proper personal hygiene. According to Standards Regarding Food Safety and Food Hygiene of Regulated Agricultural Food Products of Plant Origin destined for Export harvest workers must have access to clean hand washing equipment and clean toilets in close proximity to where harvesting is being conducted [Department of Agriculture, Forestry and Fisheries (DAFF), 2008]. Good agricultural practices require employers to train employees in basic toilet and hand washing techniques (James, 2006).

Equipment used in the field, such as crates and containers, should be cleaned on a regular basis to avoid debris build-up (James, 2006). According to Standards Regarding Food Safety and Food Hygiene of Regulated Agricultural Food Products of Plant Origin Destined for Export Act 119 of 1990, access to clean and well maintained harvesting equipment is a major control point (DAFF, 2008).

4.2. Processing Sources

Delay in transportation of freshly harvested produce to the packhouse can lead to the proliferation of any foodborne pathogens that could be potentially present, due to elevated temperatures (De Roever, 1998). Contamination in the packhouse could occur through human contact and surface contact. The Standards Regarding Food Safety and Food Hygiene of Regulated Agricultural Food Products of Plant Origin Destined for Export require that workers implement the hygiene instructions and be trained in basic hygiene (DAFF, 2008). If correct hygiene practices are in place it is important to ensure water used for hand washing and cleaning of the packhouse should be potable and not heavily contaminated. Venter and September (2006) found no significant difference in microbial load reduction when clean and moderately contaminated water was used, therefore neither would reduce microflora on the heavily contaminated hands. Venter and September (2006) found that the decontaminating factor during the hand washing procedure was the use of paper towels to dry hands.

All workers involved in distribution play an important role in preventing contamination and in maintaining the cold chain temperatures needed to ensure fruit quality and safety (Brackett, 1999; James, 2006). Shipping of produce in trucks previously used for products that could be a potential source of pathogen contamination demonstrates the importance of routine cleaning (James, 2006).

4.3. Consumer Sources

The CDC found a correlation between foodborne outbreaks and actions of consumers (CDC, 2004a). Consumers contaminate produce at the retailer level by touching fruit to test ripeness, putting unpackaged fruit into potentially contaminated shopping carts and bagging fruit with meat, fish and poultry leading to potential cross contamination (James, 2006). At the point of purchase of fresh produce, awareness and education of consumers in the correct fresh produce handling, packing and transportation procedures should be illustrated to protect the consumer from potential risk due to improper handling or abuse (Brackett, 1999; De Roever, 1998; James, 2006).

4.4. Control

Direct control methods include prevention of water and compost contamination and the maintenance of proper cold chain temperatures. Indirect waterborne contamination of produce and workers has been shown to be prevented by the treatment of water with disinfecting chemicals, filtration, ozonation, ultra-violet treatment and heat treatment (Kirby *et al.*, 2003; Steele and Odumeru, 2004). Hazard analysis and critical control points-like processes aid in the reduction of waterborne contamination in the production and processing environments (Kirby *et al.*, 2003). Preventing the access of livestock to water sources has been shown to decrease the level of water contamination by livestock (Steele and Odumeru, 2004). Contamination due to compost can be avoided by carrying out proper composting techniques to create homogeneous conditions in the compost heap by churning the heap at regular intervals (Islam *et al.*, 2005). Mulching material placed over the compost reduces contact of compost and contaminants with fruit. Mulching also prevents the indirect contamination of fresh produce by animals, like birds and insects. The maintenance of the cold chain temperatures in the production environment, processing environment and during retail is the most important aspect to prevent contamination by and amplification of foodborne pathogens.

A number of prevention measures also include the assessment of the current food safety management system and determination of the level of risk associated with specific produce. A multifaceted approach is therefore needed to control outbreaks, contamination and possible prevention of foodborne pathogen contamination (De Roever, 1998). By conducting an analysis of the stringency and efficiency of the horticultural safety management system it is possible to find ways and areas where improvement is required. Food safety management systems involve the establishment of pre-requisite programmes (Bas *et al.*, 2007), critical control points (Domenech *et al.*, 2008), an adequate reporting system (Bas *et al.*, 2007), proper compliance and traceable documentation (Azanza and Zamora-Luna, 2005) and a food safety management system (Nguyen *et al.*, 2004). In 2011, Luning *et al.* published "A tool to diagnose context of riskiness in view of food safety activities and microbiological safety output", this study was aimed at differentiating and assessing the food safety management system in levels. The assessments were developed by determining the specific microbiological output of a food safety management system, then the different distinct levels of riskiness was determined and defined where after the relationship between the levels of factors and the levels of control and assurance activities were discussed.

A microbial risk assessment will also aid in the control of possible contamination events by determining and implementing critical control points that could lead to the prevention of possible outbreaks (Doyle, 2000). A microbiological risk assessment is a structured way to assess the level of risk associated with a biological hazard in food [World Health Organisation (WHO), 1999]. The main aim of a risk assessment is to determine the likelihood of harm resulting in human exposure to agents in food. A risk assessment is associated with a certain degree of uncertainty and can be used to determine data gaps therefore allowing the improvement of the level of information available for specific hazard and product combinations. A full microbiological risk assessment involves three main steps, (1) risk analysis, (2) risk management and (3) risk communication. The research component of the microbiological risk assessment is the risk analysis section which consists of four main sections, namely (1) hazard identification, (2) exposure assessment, (3) hazard characterisation and (4) risk characterisation. All four section of the risk analysis allow researchers to characterise the level of risk of a specific hazard-product combination. Risk assessments are mainly based on toxic chemicals in food and therefore a number of problems exist when applying the same methodology to microbial pathogens. A number of data gaps exist, therefore preventing the accurate estimation required for a quantitative risk assessment (WHO, 1999).

5. Food Safety on Fresh Produce

An increase in fresh produce consumption has been observed over the past twenty years, resulting in more foodborne disease cases and outbreaks associated with fresh fruit and vegetables (Table 2.1) (Todd, 1997). Reported outbreaks on fresh produce in the United States of America have doubled from the period of 1973-1992 (104 outbreaks) (CDC, 2006a; De Roever, 1998). Mead and others (1999) reported that 76 million cases of sporadic foodborne diseases which resulted in 5 000 deaths in the USA in 1999 (Mead *et al.* 1999). Diseases can be life threatening due to the virulence factors carried by each pathogen (Table 2.2). In the United State of America, a mean average of 320 outbreaks resulting in 8 098 cases were reported from 2002 to 2006 and 259 outbreaks resulting in 7 115 cases were reported in 2007 (CDC, 2010). The increase may also be attributed to longer food chains made possible by new technologies, allowing the introduction and proliferation of pathogens affecting a larger number of people globally. This reported increase can also be attributed to the better recording of diseases internationally, the public's awareness to report diseases and the improved diagnostics that allow the identification of these pathogens. Prior to a disease outbreak, pathogen survival and proliferation may cause a risk to the end consumer.

Foodborne outbreaks are largely under reported and go unnoticed in developing countries due to the rarity of scientific investigation because of the lack of feasibility. The statistics of outbreaks that occur in developing countries are also not readily accessible as in developed countries, due to the lack of record keeping. In this review, five developing countries (South Africa, Mexico, Brazil, India and China) and five developed countries (Australia, USA, Japan, EU and Hong Kong) were compared to determine the availability of disease outbreak information. All developed countries information was readily accessible through the worldwide web, but information regarding developing countries was scarce or not available. A summary of these findings are presented in Table 2.3.

5.1. Underreported outbreaks

Outbreaks are largely underreported worldwide due to a number of reasons. Mead *et al.* (1999) took the number of unreported cases into account when reanalysing the number of outbreaks that occurred in the USA in 1999. In the USA, 73 480 *E. coli* O157:H7 infections occur per annum with 85% being transmitted by food (Mead *et al.* 1999). In Taiwan only two *E. coli* O157:H7 infections were reported in 1996 (Su *et al.,* 2005), proving the large amount of cases not reported due to mild infections and less severe symptoms requiring medical attention or reporting. Most *L. monocytogenes* infections are reported due to the severity of

Table 2.1: Summary of some reported foodborne pathogens associated with fruit crops

Fruit name	Foodborne pathogens	Product type	Ref.
Almonds	*Salmonella* Enterica	Surface	a
Apples	*Cryptosporidium parvum*	Unpasteurised apple cider	b; c; d
	Escherichia coli O157:H7	Juice and cider	e
	Escherichia coli O111	Unpasteurized apple cider	d
	Salmonella Typhimurium	Unpasteurized apple cider	f
	Norovirus	Apple Pie	g
Banana	*Salmonella* Heidelberg	Banana pudding	d; h
	Staphylococcus aureus	Banana pudding	d
	Norovirus	Banana pie	i
Berries excluding strawberries	*Cyclospora cayetanesis*	Raspberry surface	j
	Calicivirus	Frozen raspberries	k
	Hepatitis A virus	Frozen raspberries	l
Grapes	*Escherichia coli* O157:H7	Red Grapes	aa
	Salmonella Berta	Grapes	aa
	Salmonella Senftenberg	Green Grapes	aa
Grapefruit	Norovirus	Salad	m
Lemons & Limes	*Staphylococcus aureus*	Full fruit	m
	Norovirus	Lemonade	n
Mango	*Salmonella* Heidelberg	Full fruit	m
	Salmonella Newport	Full fruit	o; p; aa
	Salmonella Oranienburg	Full fruit	m; aa
	Salmonella Saint Paul	Full fruit	m; n; aa
Melons	*Bacillus cereus*	Surface	m
	Campylobacter jejuni	Melons	g
	Escherichia coli O157:H7	Cantaloupe	m
	Salmonella spp.	Cantaloupe	m; aa
	Salmonella chester	Cantaloupes	q
	Salmonella Chester	Cantaloupe	m
	Salmonella Enteritidis	Honeydew	m; aa
	Salmonella Javiana	Cut	m; aa
	Salmonella Litchfield	Cantaloupe	aa
	Salmonella Muenchen	Cantaloupe	m; aa
	Salmonella Newport	Melons	g; aa
	Salmonella Poona	Cantaloupe	m; aa
	Salmonella Saphra	Cantaloupe	m
	Shigella sonnei	Honeydew	c; m
	Staphylococcus aureus	Surface	m
	Norovirus	Cantaloupe	d; m
	Norwalk virus	Sliced	r
	Salmonella	Cantaloupe	s

Table 2.1 cont.

Fruit name	Foodborne pathogens	Product type	Ref.
Oranges	*Salmonella* Hartford	Unpasteurised orange juice	t
	Salmonella Saint Paul	Unpasteurised orange juice	i
	Salmonella Typhimurium	Unpasteurised orange juice	i
Peaches	*Escherichia coli*	Unwashed peaches	u
	Salmonella spp.	Full fruit	u
Peanuts	*Salmonella* Tennessee	Peanut butter	v
	Salmonella Thompson	Surface	v
Pears	*E. coli* O157:H7	Surface	m; aa
Pineapple	Norovirus	Surface	m; h
Plums	*Escherichia coli*	Washed plums	u
	Salmonella Newport	Surface	v
Strawberries	*Campylobacter jejuni*	-	m
	Escherichia coli O26	Surface	v; aa
	Salmonella Group B	Surface	m; v
	Hepatitis A virus	Frozen	w
	Norovirus	Surface	m; v
Table Grapes	*Escherichia coli*	Red grapes	m
	Salmonella Senftenberg	Green grapes	m
	Norovirus	Red grapes	m
Watermelon	*Escherichia coli* O157:H7	-	aa
	Salmonella Berta	-	h
	Salmonella Enteritidis	-	m; aa
	Salmonella Javiana	-	m; x; aa
	Salmonella Newport	-	aa
	Salmonella miami	Sliced watermelon	y
	Salmonella oranienburg	Pre-cut watermelon	z
	Norovirus	-	m; v

a: CDC, 2004a; b: CDC, 1997b; c: CDC, 2003; d: CDC, 2004b; e: Besser *et al.*, 1993; f: CDC, 1975; g: CDC, 2006c; h: CDC, 2002; i: CDC, 2005b; j: CDC, 1997c; k: Ponka *et al.*, 1999; l: Reid and Robison, 1987; m CSPI, 2008; n: CDC, 2001; o: Sivapalasingam *et al.,* 2003; p: Penteado *et al.,* 2004; q: CDC, 1991; r: Inversen *et al.*, 1987; s: CDC, 2008a; t: Cook *et al.*, 1998; u: Abdelnoor *et al*., 1983; v: CDC, 2006d; w: Niu *et al.*, 1992; x: Blostein, 1993; y: Gaylor *et al.,* 1955; z: CDC, 1979

Table 2.2: Four important pathogens: characteristics, disease and virulence factors

Organism	Optimal conditions for Growth	Diseases	Virulence Factors	Sources	Ref.
Escherichia coli O157:H7	Mesophile (opt. 37^{o}C), can survive refrigeration temperatures	Haemorrhagic colitis, haemolytic uremic syndrome, micro-angiopathic haemolytic anaemia and thrombotic thrombocytopenia	Verotoxins (VTI and VTII), haemolysin and locus of enterocyte effacement	Faeces, water and food handlers	a, b, c, d
Listeria monocytogenes	Psychrotroph (opt. 37^{o}C), can survive freezing temperatures	Meningitis and meningoencephalitis in pregnant women, neonates, elderly and immunocompromised. Abortion, stillbirth, pneumonia and central nervous involvement.	Listeriolysin protein, phospholipase C protein (escape vacuole), actin protein (cell-to-cell spread) and *PrfA* transcriptional regulator protein	Vegetation, sewage, soil and plant-soil rhizosphere	a, e, f; g, h, i
Salmonella spp.	Mesophile (opt. 37^{o}C), can survive freezing temperatures	Enteriditis: asymptomatic to severe diarrhoea, mild fever, nausea and vomiting, abdominal pain and diarrhoea. Systemic: Typhoid fever with fever, headache, abdominal tenderness and constipation	*Salmonella* pathogenicity island (SPI) I (invasion, intestinal secretion and inflammatory response), type II secretion system (translocation of proteins into host) and SPI II (macrophage survival, actin reorganisation and formation of *Salmonella*-induced filaments)	Sewage, faeces and water	a, j, k, l, m
Staphylococcus aureus	Mesophile (opt. 37^{o}C), can survive freezing temperatures	Cutaneous infection (Furunculosis, impetigo and abscesses), organ infections (osteomyelitis, endocarditis and arthritis), toxinoses (food poisoning, septic shock syndrome, scalded skin syndrome and toxic shock syndrome)	Surface proteins (adherence to tissue, protein build up in blood and iron uptake promotion), membrane damaging proteins, innate immune evasion proteins, enterotoxins, exfoliative toxins and toxic shock syndrome toxin	Food handlers, skin inhabitant and nosocomial infections	n, o

a: Adams and Moss, 2000; b: Mead and Griffin, 1998; c: Sears and Kaper, 1996; d: : Willshaw *et al.*, 2000; e: Bremer *et al.*, 2003; f: Farber and Peterkin, 2000; g: Jurado *et al.*, 1993; h: McLauchlin, 1990; i: Portnoy *et al.*, 1992; j: D'Auost, 2000; k: Groisman and Ochman, 1997; l: Lilić and Stebbins, 2004; m: Ohl and Miller, 2001; n: Foster, 2001; o: Novick *et al.*, 2001

Table 2.3: Summary of outbreaks in developed and developing countries

Country or region	Development status	Year or period monitored	Number of outbreaks	Number of cases	Average number of cases or outbreaks per year	Ref.
Australia	Developed	2001-2007	12423	nd	1774.7	a
USA	Developed	2001	1243	25035	1198.8	b
		2002	1330	24971		b
		2003	1072	22791		c
		2004	1319	28239		d
		2005	982	20179		e
		2006	1247	25659		f
		2007	1097	21183		G
Japan	Developed	Ni	Ni	Ni	Ni	Ni
European Union	Developed	2001-2004	Ni	Ni		Ni
		2005				h
		2006	5710	53568		i
		2007	5609	40000		j
Hong Kong	Developed	2001	671	2707	746.7	k
		2002	670	2640		k
		2003	422	2230		k
		2004	821	3131		k
		2005	927	3595		k
		2006	1095	4145		l
		2007	621	1992		l
Mexico	Developing	2001-2007	Ni	Ni	Ni	-
Brazil	Developing	2001-2007	Ni	Ni	Ni	-
South Africa	Developing	2001-2007	Ni	Ni	Ni	-
India	Developing	2001-2007	Ni	Ni	Ni	-
China	Developing	1994-2005	1082	57612	4801	m
		2006-2007	Ni	Ni	Ni	-

Ni- no information obtained during literature searches

a: Kirk *et al.,* 2008; b: CDC, 2006a; c: CDC, 2003; d: CDC, 2004a; e: CDC, 2005a; f: CDC, 2006b; g: CDC, 2007; h: European Food Safety Authority (EFSA), 2009; i: EFSA, 2007a; j: EFSA, 2007b; k: Chan and Chan, 2007; l: Hong Kong Department of Health, 2009; m: Wang *et al*., 2007

the disease with 99% associated with food (Mead *et al.,* 1999). In the USA, 2 518 cases of listeriosis occurred in 1999. An estimated 824 incidence of typhoid fever occur in the USA per year, while only 28 were reported in Taiwan in 1996 (Mead *et al.,* 1999; Su *et al.,* 2005). Staphylococcal food poisoning often goes unnoticed or unidentified therefore largely

underestimated. Mead *et al.* (1999), estimated that 185 060 events of staphylococcal infection occur in the USA, with 100% being associated with food. The highest number of reported *S. aureus* infections, in Taiwan, was in 1997 with 50 being reported (Su *et al.,* 2005).

Specific recent outbreaks of *L. monocytogenes* and *S. aureus* within the last ten years on fresh produce were not found during the reviewing of the literature. Possible reasons for the lack of information of these organisms on fruit could be that fruit rarely comes into contact with soil (*L. monocytogenes'* natural habitat) if harvesting occurs correctly, therefore the number of reported outbreaks is low. *Staphylococcus aureus* disease is due to the production of a toxin that gives mild symptoms and is often not documented because the symptoms are mild and the toxin is not necessarily produced.

5.2. *Listeria monocytogenes* outbreaks

Three documented cases of *L. monocytogenes* outbreaks were associated with fresh produce in the USA. Recently in 2011 there was a multistate outbreak of *L. monocytogenes* associated with cantaloupe in the USA (CDC, 2011). In 1986, eight Boston hospitals had a common outbreak of *L. monocytogenes* that may have been associated with lettuce or celery served with food consumed by all patients (Ho *et al.,* 1986). In 1983 coleslaw was reported to be the source of an outbreak of *L. monocytogenes,* where seven adults and 34 perinatal infections were reported (Schlech *et al.,* 1983).

5.3. *Escherichia coli O157:H7* outbreaks

Escherichia coli O157:H7 can cause severe symptoms and illnesses, therefore detection and reporting are more efficient. In 2012, there was a multistate outbreak of *E. coli* O157:H7 in the USA which infected 58 people following the consumption of contaminated Romaine lettuce (CDC, 2012). A large outbreak of *E. coli* O157:H7 occurred in September 2006 where 183 people were infected in the USA, of which 95 people were hospitalised, 29 people had haemolytic uremic syndrome and one person died (CDC, 2006b). This outbreak was linked to the consumption of bagged spinach. Two outbreaks occurred in 1997. The first was associated with alfalfa sprouts (CDC, 1997a) and the second outbreak was associated with unpasteurised apple (CDC, 1997b).

5.4. Salmonella spp. outbreaks

The most recent outbreak of *Salmonella* occurred during the period of April to August 2008. Infections were found to be linked to the consumption of raw tomatoes, jalapeño and cilantro (normally consumed together) (CDC, 2008a). A second outbreak occurred in 2008, associated with cantaloupe (CDC, 2008b). A previous outbreak in 2006 was also associated with the consumption of tomatoes, where 183 cases were reported in 21 states (CDC, 2006c). In 2004, three outbreaks of *Salmonella* were associated with Roma Tomatoes in the USA and Canada (CDC, 2005a). The outbreaks involved 561 infected people and the source of the infection was identified as the tomato packhouse, although growers might have supplied contaminated tomatoes. In May 2004 an outbreak of *Salmonella enterica* serotype Enteritidis occurred the source of infection was raw almonds. Two hundred and seven cases of *Salmonella* serotype Muenchen infection were reported throughout the USA and Canada in June 1999 (CDC, 1999). The source of the outbreak was unpasteurised orange juice traced to a single producer.

6. Conclusion

Food safety assurance is an integral part of the production of fresh produce. Assurance allows increased consumption of fresh produce that is safe and will not lead to a foodborne disease outbreak. Foodborne disease outbreaks associated with fresh produce are increasing worldwide, due to a number of factors such as extended supply chains and increased handling. In general foodborne disease outbreaks are less well recorded in developing countries and comparable statistics are not available. In order to reduce food safety risks from a microbiological perspective a risk assessment approach is essential for the whole supply chain to ensure product safety and integrity. Producers are responsible for product safety and are required to comply with multiple standards, yet such rigorous compliance is not required for the rest of the supply chain. As developing countries become more compliant with international trade standards and requirements, capacity will be developed to more effectively regulate, monitor and control foodborne pathogens. A holistic supply chain approach is therefore required to ensure product safety in a global context.

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Chapter 3

Multiplex Polymerase Chain Reaction for the detection of *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* subsp. *enterica* Typhimurium and *Staphylococcus aureus*, on artificially inoculated fresh produce

Stacey Collignon, Rene Jacobs, Carinne Joubert and Lise Korsten

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Abstract

Globally the interest in foodborne pathogens has increased due to more reported disease outbreaks, increased public awareness, a bigger proportion of the population being immunocompromised and more effective detection methods. The risk of introducing foodborne pathogens into the food chain is further increased due to the more extensive distribution systems. The effective implementation of food safety systems is thus required to prevent initial product contamination, ensure integrity and reduce the risk of foodborne disease outbreaks. Forming part of this preventative strategy is the rapid, repeatable and more cost effective detection of foodborne pathogens on fresh produce to ultimately protect the consumer. Important foodborne pathogens reported thus far on fresh fruit and vegetables are Escherichia coli serotype O157:H7, Listeria monocytogenes, Salmonella spp. and Staphylococcus aureus. The aim of this study was to develop a rapid and accurate DNA based test method to detect these pathogens on fresh produce in one reaction with a result obtained within 48 hours. Following isolation from artificially inoculated fresh produce a universal enrichment step is included to increase initial titres to a detectable level. Following enrichment of the samples and pure cultures the extracted DNA is subjected to a multiplex PCR using selected primer sets. Sensitivity of the test method ranged between 100-102 cfu/ml using individual pure cultures. Detection of E. coli O157:H7, Salmonella Typhimurium and S. aureus was possible at initial titres as low as <101 cfu/fruit on intact fruit (peaches, pears, oranges, apples, plums) and <101 cfu/g on lettuce and beans. Detection levels of L. monocytogenes were as low as 102 cfu/fruit or cfu/g. The use of a sensitive and repeatable multiplex test method, that can detect the presence of four of the most important foodborne pathogens on fresh produce, will enable government and industry to more accurately and rapidly monitor fruit and vegetables for compliance in food safety systems.

1. Introduction

Global distribution of food products requires comprehensive and complex food chains, increasing the risk of contamination due to increased handling. Poor personal hygiene and inadequate facility sanitation can lead to an increased risk of contaminating food products moving through the food chain. The risk of contamination of food with biological hazards has also increased due to a greater risk of exposure to a wider range of foodborne pathogens originating from different sources. Consumers are also now more aware of the possibility that food may be contaminated and therefore demand more stringent food safety systems. As pressure on retailers increases to ensure product safety, so will liability of all role players in the supply chain. Rapid detection of foodborne pathogens has therefore become an important requirement in the food industry to ensure that contaminated produce can be recalled in time and be removed from the food chain (Van der Vossen and Hofstra, 1996).

Escherichia coli has been detected on surfaces of plums (Abdelnoor *et al.*, 1983), strawberries (CDC, 2006) and table grapes (CSPI, 2008), *Salmonella* spp. have been detected on mango (Sivapalasingam *et al.* 2003), cantaloupe (CSPI, 2008), plums (CDC, 2006), strawberries (CDC, 2006; CSPI, 2008) and table grapes (CSPI, 2008); and *Staphylococcus aureus* was detected on lemons (CSPI, 2008) and cantaloupe (CSPI, 2008). Pathogen detection on surfaces of fruit and vegetables does not necessarily lead to contamination of a minimally or fully processed product at a high enough titre that would possibly lead to a foodborne outbreak. *Escherichia coli*, *Salmonella* spp. and *S. aureus* have been isolated from processed products such as apple juice (Besser *et al.*, 1993, CDC, 1975), orange juice (CDC, 2005) and banana pudding (CDC, 2004), respectively. Detection of these pathogens on intact fruit and vegetables or in its processed derivative does not necessarily lead to a foodborne disease outbreak but is an indication that an outbreak is possible. Since these pathogens can be associated with a variety of fresh products, it becomes essential to have a rapid, versatile and universal detection method.

Detection of foodborne pathogens was traditionally achieved by using selective cultural methods followed by pathogen identification and confirmation. These cultural methods are time consuming, laborious, non-specific and are not sensitive enough to detect low titres of pathogens present on and in different food products (Aznar and Solis, 2006; Odumeru *et al.*, 1999). To increase sensitivity of a specific methodology, a non-selective enrichment step is often included to allow general growth of all microorganisms, which may result in growth of competitive organisms presenting a false positive reaction. By including an organism's specific selective growth medium, the target organism can be selectively enriched, increasing

sensitivity and specificity of the chosen test method. Confirmation of identity is thereafter required since selective enrichment is not always sensitive and accurate. An easy microbiological identification method such as Analytical Profile Index (API)™ (Aznar and Solis, 2006) or Biolog (Odumeru *et al.,* 1999) can be used. The API™ showed a relatively low sensitivity of 67% when using the traditional cultural detection (Aznar and Solis, 2006). The Biolog system reviewed by Odumeru *et al. (*1999) was more sensitive with 72.5% sensitivity for *E. coli* with 100% accuracy, 97.5% sensitivity for *L. monocytogenes* with 100% accuracy and 95% sensitivity for *Salmonella* spp. with 100% accuracy. The use of a PCR test to confirm identity is more accurate than traditional methods. The specific primer designs for PCR increase the specificity and accuracy of a PCR test methodology. Direct PCR for individual pathogens allow confirmation and identification of a specific organism in one step. The BAX PCR detection method for *E. coli* O157:H7 tested by Shearer *et al.* (2001) on fifteen food products proved to be more sensitive than traditional cultural methods. Bennett *et al.* (1998) and Johnson *et al.* (1998) reported 96.5% sensitivity of the PCR based methodology. Polymerase chain reaction detection of microorganisms allows the detection of non-viable and living bacteria. Detection of foodborne pathogens is dependent on food matrices, processing and enrichment, for example detection of *L. monocytogenes*, *Salmonella* Typhimurium and *Campylobacter jejuni* occurred at 10^3 cfu/reaction (Wang and Slavik, 2005), whereas Zhaung and Mustapha (2005) obtained detection levels of 158 cfu/ml for *E. coli* O157:H7, *Salmonella* and *Shigella*.

Multiplex PCR has been used in different ways to detect a number of foodborne pathogens in a single reaction reducing detection time and costs (Perry *et al.*, 2007). In 2006, Park *et al*. reported a multiplex PCR for the detection of *E. coli* O157:H7, *L. monocytogenes*, *S. aureus* and *Salmonella* species. This however only allowed detection of *Salmonella* to a genus level and not specifically to serovar level. In 2005, Zhaung and Mustapha used a multiplex PCR to detect *E. coli* serotype O157:H7, *Salmonella enterica* subsp. *enterica* Typhimurium and *Shigella flexneri* due to an increase in reported foodborne disease outbreaks associated with the various pathogens in raw and ready-to-eat meat products. Wang and Slavik (2005) detected *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium and *C. jejuni* in a number of artificially inoculated meat, fruit and vegetable products. Mukhopadhyay and Mukhopadhyay (2007) developed a novel multiplex PCR with an alternative enrichment method for the detection of *E. coli* O157:H7 and *L. monocytogenes*. The alternative enrichment method was developed because *E. coli* O157:H7 was a more effective competitor in media than *L. monocytogenes*.

In this study a multiplex PCR was developed for the detection of four of the most important foodborne pathogens *i.e. E. coli* serotype O157:H7, *L. monocytogenes, S. enterica* subsp. *enterica* and *S. aureus* isolated from fresh produce. The method was optimized to ensure effective and accurate detection from fresh produce.

2. Materials and Methods

2.1. Bacterial Strains

American Type Culture Collection (ATCC, Manassas, United States of America) cultures [*E. coli* O157:H7 (ATCC 35150), *L. monocytogenes* (ATCC 19115), *Salmonella* Typhimurium (ATCC 14028) and *S. aureus* (ATCC 12600)] were used in this study. All cultures were maintained, lyophilised and stored at -70^{o}C with subcultures on standard 1 medium (Merck, Johannesburg, South Africa) prepared 24 hours prior to use. All research was done in a laboratory that is managed under the ISO 17025 laboratory management system.

2.2. Universal Broth for the Culturing of Pathogens

Four readily available universal broth media namely buffered peptone water (PBW), nutrient broth (NB), tryptone soy broth (TSB) and tryptose broth (TB) (all from Merck), were tested and compared for their ability to best support the growth of the four foodborne pathogens individually *i.e. E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* on their own. Each of the broths (100 ml) was inoculated using one 24 hour old colony per broth (in triplicate). Broths were shake incubated (100 rpm) at 37^{o}C for 48 hours and subsequently analysed for cell density using an Ultraspec 4050 (LKB Biochrom, UK) spectrophotometer operating at 620 nm using six measurements per organism per broth per repeat. The experiment was repeated and the most effective broth was identified based on enhanced growth of all four organisms individually.

One colony of each pathogen was inoculated into 100 ml of the same selected broth *i.e.* TSB (in duplicate) to determine the combined effect that the pathogens have on one another when enriched together. Inoculated broths were shake incubated (100 rpm) at 37^{o}C for 18 hours. A dilution series was prepared with subsequent plating on the corresponding selective medium *i.e.*, Baird-Parker agar for *S. aureus*, Oxford Listeria Selective agar for *L. monocytogenes*, MacConkey agar with crystal-violet for *E. coli* O157:H7 and XLD agar (all supplied by Merck) for *Salmonella* Typhimurium, the number of colony forming units (cfu) were recorded and transformed to log (x+1) cfu/ml. This experiment was repeated twice.

2.3. DNA Extraction

The DNA extractions performed were done using the 1% (v/v) Triton X-100 (Sigma, Johannesburg) method (Wang and Slavik, 2005) with modifications. Mixed pathogens were cultured (shake incubated at 100 rpm and 37^{o}C for 48 hours) in TSB (9ml), followed by centrifugation at 6 000 g for 5 minutes. Broth medium was removed by repetitive washing of the pellet with sterile water, followed by centrifugation before final resuspension in 50 µl 1% (v/v) Triton X-100. Extracted DNA was visualised by electrophoresis on a 1 % (w/v) agarose (Bio-Rad, USA) gel containing 0.1% (v/v) Ethidium Bromide (10mg/ml) (Merck) and visualised with a Vilber Lourmat Gel Documentation System and digitally imaged using E-capt software (both supplied by Vilber Lourmat, Marne La Valle, France) to ensure that the DNA extracted from all samples was of similar quantity.

2.4. Primer Design

Primers were designed for *S. enterica* subsp. *enterica* and *S. aureus* using Primer 3 software (Rozen and Skaletsky, 2000). *Salmonella enterica* subsp. *enterica* primers (SLDF and SLDR, Table 3.1), targeting the long polar fimbriae D gene (U18559, GenBank database) were designed using the sequence obtained from the GenBank database. *Staphylococcus aureus* primers (SN2F and SN2R, Table 3.1) were designed targeting the staphylococcal nuclease gene (DQ 507382, GenBank database) with sequences obtained from the GenBank database. Homology studies were performed against the GenBank database for sequence similarity using NCBI BLAST.

Table 3.1: Primers used for the detection of *Escherichia coli* O157:H7*, Listeria monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus*

Microorganism	Primer	Primer sequence 5'-3'	Size Bp.	Ref.
Escherichia coli O157:H7	UidAa	GCG AAA ACT GTG GAA TTG GG	252	¥
	UidAb	TGA TGC TCC ATA ACT TCC TG		
Listeria monocytogenes	LMFP	AGC TCT TAG CTC CAT GAG TT	450	*
	LMRP	ACA TTG TAG CTA AGG CGA CT		
Salmonella Typhimurium	SLDF	CCT GTG AAT GCC CTG ATG AT	787	#
	SLDR	TTG CCG GTG GTA CTG ATA GG		
Staphylococcus aureus	SCN2F	TTG CAT ATG TAT GGC AAT TGT T	655	#
	SCN2R	TTT TGC TTG TGC TTC ACT TTT TC		

¥ Cebula *et al.* 1995; * Goldsteyn Thomas *et al.*, 1991; # This study

2.5. Multiplex PCR

One colony of *E. coli* O157:H7, *L. monocytogenes*, *S. enterica* subsp. *enterica* and *S. aureus* was used to inoculate TSB (9ml) individually and as a pathogen mix. Inoculated broths were shake incubated at 150 rpm for 18 hours at 37^{o}C. The extraction and PCR of the individual cultures and pathogen mix was performed in duplicate and the whole experiment was repeated three times. A 25 µl PCR reaction mix was used which included the following: 0.3 µl of BioTaq polymerase (5U/µl), 1.5 µl $MgCl_2$ (50mM), 0.5 µl dNTPs (10mM of each), 2.5 µl NH_4 reaction buffer (10x) (all from Bioline, Celtic Molecular Diagnostics, Cape Town, SA), 0.75 µl Bovine Serum Albumin Acetylated (10 mg/ml) (Promega, Madison, USA), 1.25 µl Dimethyl Sulfoxide (Saarchem, Merck), 0.3 µl of each primer at optimal concentration [30 pmol UidAa and UidAb, 20 pmol LMFP and LMRP, 60 pmol SN2F and SN2R and 50 pmol SLDF and SLDR (Invitrogen, Johannesburg), Table 3.1] and 1.5 µl extracted DNA. A Mastercycler ep (Eppendorf, Germany) thermocycler was used with the following cycling conditions: an initial denaturation at 95^{o}C for 2 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 61°C for 45 seconds and extension at 72°C for 90 seconds, with a final extension at 72°C for 7 minutes. The PCR amplicon was visualised (section 2.3.) on a 2% (w/v) agarose gel containing 0.1 % (v/v) Ethidium Bromide and visualised with a Vilber Lourmat Gel Documentation System and digitally imaged using E-capt software.

2.6. Detection levels in Pure Culture

An overnight culture (18 hours at 37^{o}C, shake incubated) (10^8 cfu/ml) of each pathogen (*E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus*) was grown individually in TSB. After incubation the cultures were separately diluted to achieve concentrations of 10^0, 10^1 and 10^2 cfu/ml for each pathogen. The concentration of bacteria was confirmed by subsequent dilution series and plating onto selective agar, as in section 2.2, with counts recorded after 24 hours incubation at 37^{o}C. One millilitre of the diluted individual cultures (10^0,10^1 and 10^2 cfu/ml) were then used to inoculate 9 ml TSB with a subsequent incubation at 37^{o}C for 48 hours. Incubated TSB was then used to perform a 1% (v/v) Triton X-100 DNA extraction (section 2.3). A multiplex PCR was performed on DNA extracted with conditions as described in section 2.5. Polymerase chain reaction amplicons were visualised as in section 2.5. The experiment was performed twice and the average log cfu/ml obtained to determine the lowest detectable levels for each pathogen.

2.7. Fresh produce pathogen detection on artificially contaminated fruit and vegetables

Seasonal fruit namely; oranges, apples, pears, plums and peaches; and vegetables; namely lettuce and green beans were all purchased from a local retailer in Pretoria, South Africa. Fresh produce selected for this study were of uniform size and weight and without pest, disease or damage. The outline of this section is illustrated in Figure 3.1.

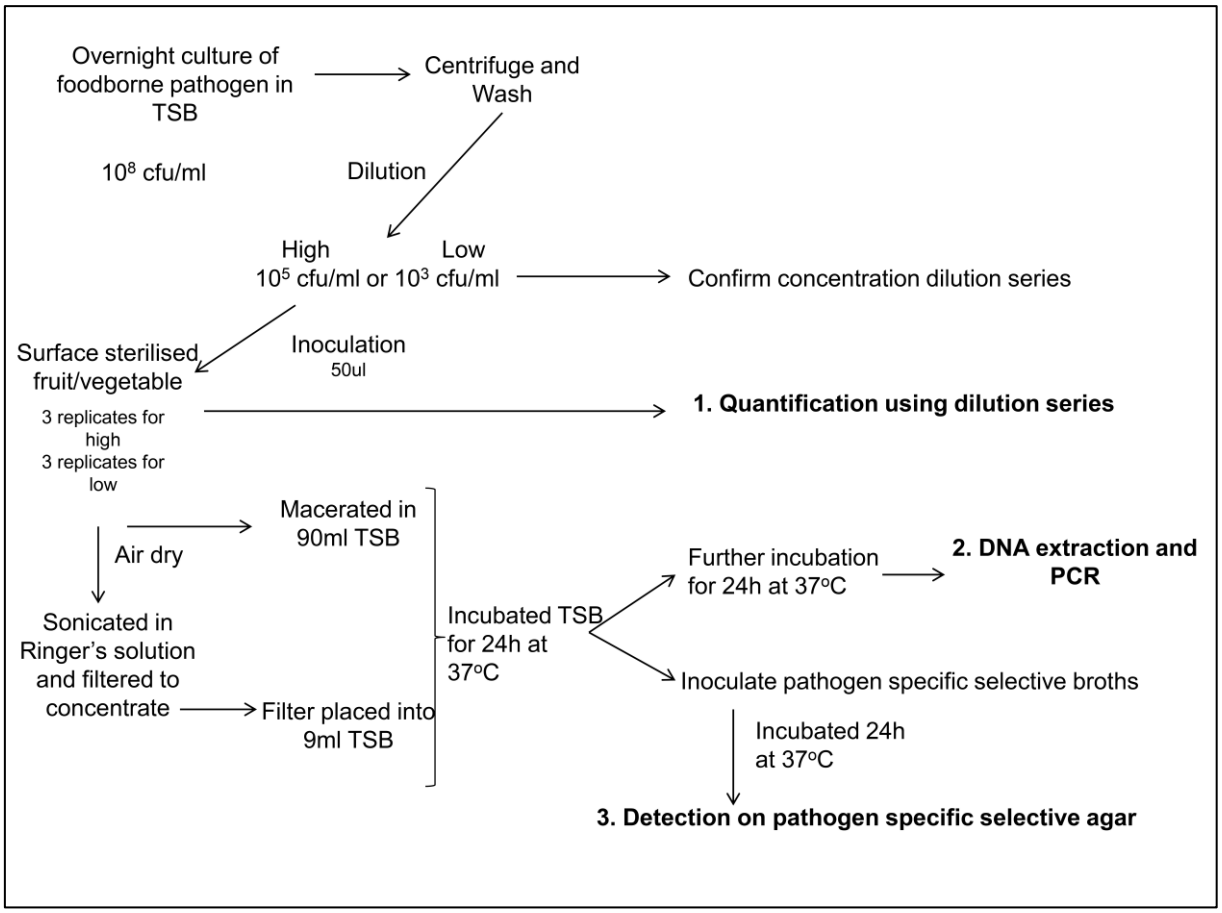

Figure 3.1: An illustration of the culture preparation, dilution, quantification and detection scheme for the fresh produce pathogen detection on artificially contaminated fruit and vegetables.

The three important aspects of the study are numbered, 1: The quantification of pathogens present on fruit or vegetables after inoculation and air dry and prior to enrichment, 2: DNA extraction and PCR from the sample TSB used in this study for the detection using pathogen specific selective broth and agar (3).

An overnight culture of each pathogen (*E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus*) was grown individually in triplicate in 100 ml TSB (Merck) Cultures were centrifuged at 5000 rpm and washed twice with sterile distilled water and finally resuspended into 1% (w/v) PBW (Merck). Cultures were subsequently serially diluted

to obtain a high inoculum concentration of 5 log cfu/ml, and a low inoculum concentration of 3 log cfu/ml. Concentrations were confirmed by serial dilution and subsequent plating in duplicate. Fresh produce were surface sterilized using 70% ethanol (Spurr, 1979) dip treatment for 30 seconds followed by air drying prior to spot inoculation. Fruit were inoculated per whole fruit and vegetables were inoculated per 10 grams. Six of each fresh produce purchased for the study were artificially spot inoculated, three for high titre inoculation and three for low titre inoculation. Fruit and vegetables were spot inoculated using 50 µl of each respective pathogen culture and contact of the individual spots was avoided. Inoculated fresh produce was allowed to air dry in a laminar flow cabinet. Fresh produce (fruit and vegetables) inoculated with the high concentration had a final load of 3.7 log cfu/fresh produce for the high concentration and 1.69 log cfu/fresh produce for the low concentration.

Fruit were subsequently washed in 500ml quarter strength Ringer's solution amended with 0.02% Tween-80 (Sigma, Johannesburg) in the Ultrasonic Bath (Labotec, Johannesburg) for 30s. The Ringer's solution was subsequently filtered through a 0.45nm nitrocellulose membrane. After filtration the membrane was subsequently used for serial dilution, with the first tube being 9 ml TSB, (used for traditional cultural detection as outlined below and PCR detection), with the rest of the dilution series following in 9 ml Ringer's solution. The inoculated 10 g bean and lettuce samples were placed in 90 ml TSB and macerated for 2 minutes at 150 rpm, with a subsequent serial dilution. Quantification of pathogens was conducted by plating the serial dilution in duplicate onto pathogen specific selective agar to quantify the number of organisms present from the sonicated or macerated fresh produce, in order to determine the detection limits of the PCR and cultural methods. Quantification was conducted as in section 2.2 and counts recorded.

The TSB from fruit washes (9ml) and vegetable macerations (90ml) was then used for two different processes, one for traditional cultural detection using selective enrichment broths specific for each pathogen and secondly for the universal enrichment prior to PCR. The TSB was first incubated at 37^{o}C for 24 hours as a pre-enrichment step. One millilitre of the 24 hour incubated TSB was transferred into the pathogen specific selective enrichment broths for all four pathogens [Brila broth for the selection of *E. coli,* Listeria selective broth for *L. monocytogenes*, Salmonella enrichment broth according to Rappaport for *Salmonella* spp. and Staphylococcus enrichment broth for enrichment of *Staphylococcus aureus* (all broths were supplied by Merck)]. These inoculated selective broths were subsequently incubated for 24 hours at 37^{o}C. Following incubation, the pathogen specific selective broths were

streaked out onto selective agar as in section 2.2. Following 24 hours incubation 37^{o}C, agar plates were then evaluated for the presence or absence of the four inoculated pathogens.

The 24 hour incubated TSB that was used for inoculation of the pathogen specific selective broths was then incubated for a further 24 hours 24 hours at 37^{o}C (resulting in a total of 48 hours incubation of the TSB). This 48 hour incubated TSB was then used for DNA extraction (section 2.3) and PCR detection (section 2.5). A positive control of a pathogen mix was included to ensure that the DNA extraction and PCR methodology was working correctly. Detection limits were expressed as the original titres that were quantified from fruit prior to the enrichment process.

2.8. Statistical analysis

Statistical analysis was performed on cell density using the absorbance values for the broths (section 2.2) and on the log cfu/ml values for the comparison of growth as a pure and mixed culture. Statistical analysis on the repeated trials was done using GenStat for Windows Discovery Edition 2 Software (VSN International Ltd., Lawes Agricultural Trust, UK). The experiments were designed as a completely randomized design, replicated. A one-way analysis of variance (no blocking) was used to determine the difference in cell density in the different broths and the difference in growth rates in a pure culture and in the pathogen mix. Means were analysed using the least significant difference at a 1% level of significance.

3. Results

3.1. Universal Broth for the Culturing of Pathogens

The only medium that allowed equal growth with no statistical difference ($P \leq 0.01$), of all four foodborne pathogens, was TSB (Table 3.2). Buffered peptone water, NB and TS were not suitable for the growth of *L. monocytogenes* which had significantly lower cell density than the other pathogens in the test medium ($P \leq 0.01$). All pathogens grew equally well in TSB on their own when compared to growth in TSB as a mixed culture, demonstrating no significant difference ($P \leq 0.01$).

Table 3.2: *Escherichia coli* serotype O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serotype Typhimurium and *Staphylococcus aureus* subsp. *aureus* growth in broths at 620 nm absorbance

Organism	Buffered Peptone Water	Tryptose Broth	Tryptone Soy Broth	Nutrient Broth
Escherichia coli O157:H7	1.36 a	1.61 a	1.80 a	1.48 a
Listeria monocytogenes	0.21 b	0.32 b	1.68 a	0.20 b
Salmonella Typhimurium	1.36 a	1.64 a	1.90 a	1.61 a
Staphylococcus aureus	1.54 a	1.95 a	2.09 a	1.57 a

[a]: Values followed by the same letter are not significantly different.

3.2. Multiplex PCR

The multiplex PCR performed on the DNA extracted from the pathogen mixture *i.e.* containing all four pathogens yielded a banding pattern containing four amplicons of 252, 450, 655 and 787 bp in size (Figure 3.2: lanes 2 and 3). Individual cultures used for DNA extraction yielded the correct amplicon sizes for *E. coli* O157:H7 (252 bp), *L. monocytogenes* (450 bp), *S. enterica* subsp. *enterica* (787bp) and *S. aureus* (650 bp) after PCR amplification (Figure 3.2: lane 4-7). The multiplex PCR performed on the negative control yielded no amplicons, demonstrating no cross contamination (Figure 3.2: lane 8).

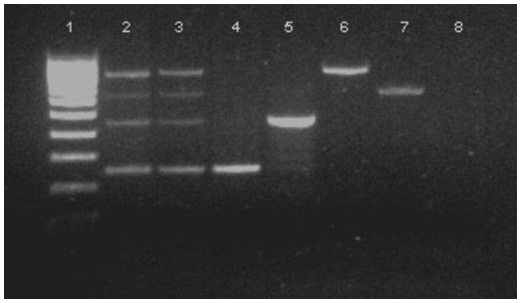

Figure 3.2: Multiplex Polymerase Chain Reaction for the detection of *Escherichia coli* serotype O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus.*

The multiplex PCR was performed in duplicate using DNA extracted from a mixed culture of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* Typhimurium and *Staphylococcus aureus* and of individually cultured *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* serovar Typhimurium and *Staphylococcus aureus*. Lane 1: 100 bp marker; Lanes 2-3: multiplex PCR, Lane 4 *Escherichia coli* O157:H7, Lane 5: *Listeria monocytogenes*. Lane 6: *Salmonella enterica* subsp. *enterica*, Lane 7: *Staphylococcus aureus* and Lane 8: negative control.

3.3. Detection levels in Pure Culture

Detection of *E. coli* O157:H7 and *Salmonella* Typhimurium occurred at all concentrations tested from an initial concentration in pure culture of 10^0 to 10^2 cfu/ml (Figure 3.3: lane 2-5; lane 10-13). The exact concentration of *E. coli* O157:H7 detected prior to enrichment was 2.6 x10^2 cfu/ml (lane 2), 5 cfu/ml (lane 3) and < 5 cfu/ml (lane 5) in pure culture and 4.3 x 10^2 cfu/ml (lane 10), 5.5 x 10 (lane 11), <5 cfu/ml (lane 12 and 13) of *Salmonella* Typhimurium was detected in pure culture (Figure 3.3). *Listeria monocytogenes* was detected from 4.3 x 10^2 cfu/ml to 5 cfu/ml prior to enrichment with a detection limit of 5 cfu/ml in pure culture (Figure 3.3: lane 6-9). *Staphylococcus aureus* had a detection limit of 10 cfu/ml in pure culture. All amplicons generated were of the correct size namely 252 bp for *E. coli* O157:H7 (Figure 3.3: lane 2 to 5), 450 bp for *L. monocytogenes* (Figure 3.3: lane 6 to 9), 787 bp for *S. enterica* subsp. *enterica* (Figure 3.3: lane 10-13) and 650 bp for *S. aureus* (Figure 3.3: lane 14-17). No amplification occurred in lane 18 (Figure 3.3) which represents the negative control.

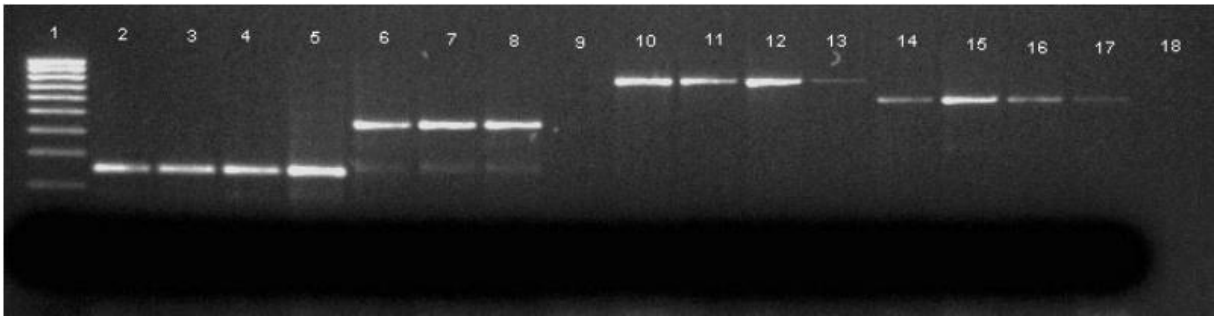

Figure 3.3: Determination of *Escherichia coli* serotype O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* lowest detection level.

The multiplex PCR was performed and run in an agarose gel. Lanes 2 to 5 represent *Escherichia coli* O157:H7, lane 6 to 9 represent *Listeria monocytogenes*, lane 10 to 13 represents *Salmonella enterica* subsp. *enterica* serovar Typhimurium and lane 14 to 17 represents *Staphylococcus aureus* and lane 18 represents a negative control. The original concentration (indicated as cfu/ml), determine by plating onto selective media, was as follows each lane: Lane 2: 2.6x10^2, Lane 3: 2x10, Lane 4: 5, Lane 5: < 5 cfu/ml, Lane 6: 4.3x10^2, Lane 7: 6.5x10, Lane 8: 5, Lane 9: < 5 cfu/ml, Lane 10: 4.3x10^2, Lane 11: 5.5x10, Lane 12: < 5 cfu/ml, Lane 13: < 5 cfu/ml, Lane 14: 1.95x10^2, Lane 15: 1x10, Lane 16: 1.5x10 and Lane 17: 1.0x10. Lane 1 represents the 100bp ladder and Lane 18 represents the negative control.

3.4. Pathogen detection on artificially inoculated fruit and vegetables

Escherichia coli O157:H7*, L. monocytogenes, Salmonella* Typhimurium and *S. aureus* were re-isolated and detected from artificially inoculated (high titre) fruit at a concentration

between <10 and 500 cfu/fruit, <10 and 183 cfu/fruit, 183 and 917 cfu/fruit and 150 cfu/fruit, respectively (Table 3.3). *Escherichia coli* O157:H7*, L. monocytogenes, Salmonella* Typhimurium and *S. aureus* were re-isolated and detected at 157 and 188 cfu/g, 193 and 353 cfu/g, 378 and 383 cfu/g of and 88 and 267 cfu/g, respectively when inoculated with high concentrations onto lettuce and beans (Table 3.3). All pathogens inoculated at high concentrations, were detected using the selective enrichment and plating methods as well as using the multiplex PCR (Table 3.3). All *E. coli* O157:H7, *Salmonella* Typhimurium and *S. aureus* were detected on fruit and vegetable sample surfaces when inoculated at high concentrations. *Listeria monocytogenes* artificially inoculated at a high concentration onto beans (353 cfu/g), oranges (<10 cfu/fruit) and plums (33 cfu/fruit) were not detected using the multiplex PCR (Table 3.3).

Salmonella Typhimurium was detected equally well when using the multiplex PCR and the traditional cultural method (Table 3.4). *Escherichia coli* O157:H7 on peaches (sample 3) and oranges (sample 2) after low inoculation was detected using the multiplex PCR but was not detected using the traditional cultural method (Table 3.4). *Listeria monocytogenes* was detected on plum (sample three) only using the multiplex PCR (Table 3.4). *Listeria monocytogenes* which was present in <10 cfu/fruit or <10 cfu/g were detected using the traditional cultural method and not the multiplex PCR on peaches (two of three samples), oranges (one of three samples), apples (two of three samples), lettuce (two of three samples) and plums (one of three samples) (Table 3.4). On peaches (one of three samples), oranges (three of three samples), apples (two of three samples), lettuce (two of three samples) and plums (one of three samples) *S. aureus* was detected using traditional cultural detection methods but not using the multiplex PCR (Table 3.4).

Escherichia coli O157:H7, *Salmonella* Typhimurium and *S. aureus*, that was present at varying titres following inoculation at a low concentration was detected on all commodities when using the multiplex PCR (Table 3.4). Detection of *L. monocytogenes* required at least 100 cfu/fruit (Table 3.4), but *L. monocytogenes* present at 353 cfu/g on beans was not detected using the multiplex PCR (Table 3.3). All four pathogens that were artificially inoculated onto plums using a low cell concentration were detected on plums using multiplex PCR and traditional cultural methodology when counts were under 10 cfu/fruit (Table 3.4). *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *S. aureus* were detected at levels below 10 cfu/fruit on peaches and pears and at below 10 cfu/g on lettuce after low concentration inoculation using both the multiplex PCR and selective enrichment and plating methods (Table 3.4). *Listeria monocytogenes,* however was not detected using the multiplex PCR following artificial inoculation with low titre inoculums (Table 3.4).

Table 3.3: Species quantification, multiplex PCR and traditional detection of *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* used at high concentrations to artificially inoculate peaches, pears, beans, oranges, apples, lettuce and plums

Method	Organism	Fresh Produce Tested at High Concentration						
		Oranges	Apples	Pears	Plums	Peaches	Beans	Lettuce
Quantification[y]	*Escherichia coli* O157:H7	500	400	33	ND	433	188	157
	Listeria monocytogenes	ND[x]	67	ND	33	183	353	193
	Salmonella Typhimurium	883	917	284	183	617	383	378
	Staphylococcus aureus	1633	333	334	150	1400	88	267
	Unit	cfu/fruit	cfu/fruit	cfu/fruit	cfu/fruit	cfu/fruit	cfu/g	cfu/g
Detection using mPCR	*Escherichia coli* O157:H7	+[z]	+	+	+	+	+	+
	Listeria monocytogenes	-[z]	+	+	-	+	-	+
	Salmonella Typhimurium	+	+	+	+	+	+	+
	Staphylococcus aureus	+	+	+	+	+	+	+
Detection using selective enrichment and plating	*Escherichia coli*	+	+	+	+	+	+	+
	Listeria spp.	+	+	+	+	+	+	+
	Salmonella spp.	+	+	+	+	+	+	+
	Staphylococcus aureus	+	+	+	+	+	+	+

[x] ND: Indicates that the organism was not detected using direct plate counts, therefore were present in numbers below 10cfu.

[y] Average cfu/fruit or cfu/g for the quantification of all three replicate.

[z] + indicates the detection of the relevant organism from all three samples of the specific commodity and - Indicates that the organism was not detected from all three samples of the commodity.

Detection of *E. coli* O157:H7, *Salmonella* Typhimurium and *S. aureus* on beans was possible when organisms were present at or below 10 cfu/g (Table 3.4). *Listeria monocytogenes* was however not detected when present between 10 and 20 cfu/g (Table 3.4). All four pathogens inoculated at low concentrations were detectable from apples when using the multiplex PCR, with *E. coli* O157:H7 and *Salmonella* Typhimurium present at fewer than 10 cfu/fruit, *S. aureus* present at 225 cfu/fruit and *L. monocytogenes* present at 800 cfu/fruit (Table 3.4). All pathogens were detected on all fresh produce using selective enrichment and plating after inoculation with low concentrations, except for *L. monocytogenes* that was not detectable on pears (Table 3.4).

Table 3.4: Species quantification, multiplex PCR and traditional detection of *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* used at low concentrations to artificially inoculate peaches, pears, beans, oranges, apples, lettuce and plums

Methods	Organisms	Sample Number	Fresh Produce Tested at Low Concentration[a]						
			Oranges	Apples	Pears	Plums	Peaches	Beans	Lettuce
Quantification	***Escherichia coli* O157:H7**	*1*	ND [x]	ND	ND	ND	300	ND	ND
		2	2950	ND	ND	ND	ND	20	95
		3	650	ND	300	ND	ND	80	30
	Listeria monocytogenes	*1*	ND	ND	ND	ND	100	10	ND
		2	ND	800	ND	ND	ND	20	ND
		3	ND	ND	ND	ND	ND	20	10
	***Salmonella* Typhimurium**	*1*	ND	ND	ND	ND	800	10	ND
		2	200	ND	ND	ND	ND	10	10
		3	ND	ND	ND	ND	ND	10	15
	Staphylococcus aureus	*1*	100	300	100	ND	100	10	30
		2	ND	100	ND	ND	ND	20	ND
		3	100	150	ND	ND	ND	ND	10
	Unit		**cfu/fruit**	**cfu/fruit**	**cfu/fruit**	**cfu/fruit**	**cfu/fruit**	**cfu/g**	**cfu/g**
Detection using mPCR	***Escherichia coli* O157:H7**	*1*	+ [y]	- [y]	-	-	+	+	+
		2	+	+	+	+	+	+	+
		3	+	+	+	-	+	+	+
	Listeria monocytogenes	*1*	-	-	-	-	+	-	-
		2	-	+	-	-	-	-	-
		3	-	-	-	+	-	-	-
	***Salmonella* Typhimurium**	*1*	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+
		3	+	+	+	+	+	+	+
	Staphylococcus aureus	*1*	-	+	+	+	+	+	-
		2	-	-	+	-	+	+	+
		3	-	-	+	+	-	+	-
Detection using traditional method	***Escherichia coli***	*1*	+	+	-	-	+	+	+
		2	-	+	+	+	+	+	+
		3	+	+	+	+	-	+	+
	***Listeria* spp.**	*1*	-	+	-	+	+	+	+
		2	+	+	-	-	+	+	+
		3	-	+	-	-	+	+	-
	***Salmonella* spp.**	*1*	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+
		3	+	+	+	+	+	+	+
	Staphylococcus aureus	*1*	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+
		3	+	+	+	+	+	+	+

[x] ND: Indicates that the organism was not detected using direct plate counts, therefore were present in numbers below 10cfu.

[y] + indicates the detection of the relevant organism from all three samples of the specific commodity and - Indicates that the organism was not detected from all three samples of the commodity.

4. Discussion

The multiplex PCR developed in this study is the first species specific PCR reported for this group of foodborne pathogens in one reaction. Primer sets that are highly sensitive for *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* were used in this multiplex PCR. Several studies (Jofré *et al.*, 2005; Lang *et al*., 1994; Li and Mustapha, 2004; Park *et al*., 2006; Ramesh *et al*., 2002; Shearer *et al*., 2001; Wang and Slavik, 2005; Zhaung and Mustapha, 2005) reported on multiplex PCR that were developed for detecting various combinations of foodborne pathogens on meat (Jofré *et al*., 2005; Wang and Slavik, 2005; Zhaung and Mustapha, 2005), vegetables, fruit and derivatives thereof (Li and Mustapha, 2004; Park *et al*., 2006; Shearer *et al*., 2001; Wang and Slavik, 2005), milk and dairy products (Ramesh *et al*., 2002; Wang and Slavik, 2005) and water (Lang *et al*., 1994). The method used in this study was developed to detect a specific combination of pathogens (foodborne species and serovars) not previously targeted.

Of the four primer sets used in this study for the multiplex PCR, two were novel and specifically designed for the detection of *Salmonella* Typhimurium and *S. aureus*. Perry *et al*. (2007), stated that the accuracy of a PCR is determined by primers used for the detection of the specific organisms. By developing two new primer sets, the specificity of the multiplex PCR could be enhanced. This is also the first report to the authors' knowledge that targets the long polar fimbriae gene of *Salmonella* Typhimurium for the specific detection of the serovar. The successful detection of *S. aureus* was achieved by targeting the nuclease protein of the organism. Primers targeting the *S. aureus* clumping factor developed by Stephan *et al*. (2001) proved to be unsuccessful in this study (data not shown). Other studies also reported on the targeting of the nuclease gene in *S. aureus* for the diagnostic detection, identification and confirmation of the species (Alarcon *et al*., 2006, Brakstad *et al*., 1992, DTU Food, 2009, Kim *et al*., 2001, Kumar *et al*., 2009, Martin *et al*., 2003, Tamarapu *et al*., 2001, Wilson *et al*., 1991).

Primers used for the successful detection of *E. coli* O157:H7 was previously designed to target the altered β-glucuronidase gene of *E. coli* O157:H7 (Cebula *et al*., 1995; Lang *et al*., 1994). The primer pair targets a unique single base pair mutation in the β-glucuronidase gene of *E. coli* O157:H7 (Feng and Monday, 2000). Numerous authors have shown the specific detection of *E. coli* O157:H7 using this primer set (Cebula *et al*., 1995; Feng and Monday, 2000; Lang *et al*., 1994; Vernozy-Rozand *et al*., 2000). *Listeria monocytogenes* primers used in this study targeted the listeriolysin O gene (Mengaud *et al*., 1989) and allowed successful amplification and detection of the organism. Listeriolysin O is one of the

most common targets for detection of *L. monocytogenes.* Amagliani *et al*. (2004), Goldsteyn Thomas *et al*. (1991), Hudson *et al*. (2001), Nogva *et al*. (2000) and Rodriguez-Lazaro *et al.* (2004) all used this target gene in their study. The success of this multiplex PCR for the detection of the four important foodborne pathogens is in the combination of primers that have been successfully designed previously and in this study.

According to the AOAC, sensitivity is an important performance indicator for qualitative methods allowing an assessment of a test method (Feldsine *et al.*, 2002). The inoculum loads used in this study were lower than the AOAC recommendation for low (25-225 cfu/g) and high (250-6250 cfu/g) inoculum loads (Feldsine *et al.,* 2002). Perry *et al.* (2007), highlighted the importance to determine the sensitivity of a test method based on PCR. In this study, the sensitivity of the test method was determined using pure culture and artificially inoculated fresh produce samples. Perry *et al.* (2007), reported that primers tested by the European Commission, Food PCR project, proved that the uniplex PCRs developed by Roth *et al.* (2003) for *E. coli* O157:H7, D'Agostino *et al.* (2004) for *L. monocytogenes* and Malorny *et al.* (2003) for *Salmonella* spp. were the most sensitive of the primers tested. The PCRs had detection levels of 10^1 cells per reaction for *E. coli* O157:H7, 3-10 cells per reaction for *L. monocytogenes* and 5-50 cells per reaction for *Salmonella* spp. These results are comparable to the level of sensitivity found in this study. In general, uniplex PCR tests are more sensitive than multiplex PCR systems highlighting the sensitivity of the test developed in this study (Hsih and Tsen, 2001; Ramesh *et al*., 2002). Detection levels for laboratory culture of *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium and *S. aureus* was 5 cfu/ml.

The results obtained in this study confirm the high sensitivity of the PCR with some samples demonstrating detection using mPCR but no detection using the cultural detection method. *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *S. aureus* where detected on artificially inoculated fresh produce at levels of <10 cfu/fruit or <10 cfu/g. Li and Mustapha (2004) found similar detection thresholds when testing their multiplex PCR in apple cider, cantaloupe, lettuce, tomato and watermelon. The detection threshold for *L. monocytogenes* from pathogen artificially inoculated fresh produce in this study was 100 cfu/g or 100 cfu/fruit. Miyahara (2005) developed a multiplex PCR to detect *L. monocytogenes* and *Salmonella* spp. in various food products. It was reported that the detection threshold for *L. monocytogenes* was 395 cfu per 25 g sample, whereas *Salmonella* spp. was 2.4 cfu per 25 g sample. In the current study, 353 cfu/g of *L. monocytogenes* was not detected from beans using the multiplex PCR. Therefore, both studies demonstrated a requirement for a higher titre of *L. monocytogenes* in order for detection to be possible.

In this study, the sensitivity, specificity and optimisation of enrichment broth media confirmed the usefulness of this technique. The inclusion of an enrichment process decreases the detection limit of a PCR (Koch *et al*., 1998). The choice of enrichment method also impacts on the sensitivity of a PCR. A universal broth media is needed to sustain growth equally well of all four pathogens. *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *L. monocytogenes* and *S. aureus* have different nutritional requirements as is evident in the differing results obtained for the four broths studied. It was further found that TSB supported the growth equally well for all four pathogens. In previous studies it was found that tryptone and enrichment media are PCR inhibitors (Lantz *et al*., 1998; Rossen *et al*., 1992) this was found not to be the case in this study, emphasizing the importance of the DNA extraction wash steps. Jofré *et al.* (2005), determined the best enrichment needed for *L. monocytogenes* was a two-step system, consisting of two separate enrichment broths. The use of TSB can eliminate the need for two separate broths and will reduce time required for enrichment. Enrichment broth choices also impact on the sensitivity of the PCR as seen in the study done by Miyahara (2005). In their study, PBW was described as a poor enrichment medium for *L. monocytogenes* which has also been demonstrated in this study. Erdogan *et al.* (2002) used NB as a storage medium for *L. monocytogenes* without reporting an increase in cell numbers. When *L. monocytogenes* is grown in NB at optimal growth temperature, the increase in numbers was found to be insignificant (Erdogan *et al*., 2002). They showed that NB cannot be used as an enrichment broth for a detection method to determine the presence of *L. monocytogenes*.

In conclusion, this is a first report using a multiplex PCR that offers a reliable, robust, repeatable, sensitive and specific technique for the detection of four important foodborne pathogens on fresh produce. *Listeria monocytogenes* was the determining factor for the choice in enrichment broth media. Tryptone soy broth is nutritionally richer than other broths tested, and therefore allows for the optimal growth of all four pathogens when used on its own or as a mixed culture. The universal broth media allows for the detection of the four pathogens in pure culture and when directly isolated from fresh produce. Further studies should investigate the possible decrease in enrichment time, without loss in method sensitivity and specificity. This method provides a robust testing tool that can be used for food safety compliance in the fresh produce industry.

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Chapter 4

Assessment of possible foodborne pathogen presence on peaches and in the production and postharvest environments

Stacey Collignon and Lise Korsten

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Abstract

Contamination of fruit with foodborne pathogens can occur through various ways and at different points within the supply chain while the fruit move from farm to retail. Foodborne pathogens are able to attach to and colonise the peach fruit surface and survive the local and export supply chains. It is therefore important to implement preventative strategies and ensure effective food safety management systems The aim of this study was to determine the presence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Staphylococcus aureus* in water, and on fruit and contact surfaces in the production arena to determine the various potential sources of contamination. Fruit, water and contact surfaces were collected from two peach farms in Limpopo and Western Cape Provinces and analysed using pathogen enrichment step followed by DNA extraction and multiplex PCR. A total of 534 samples were collected and analysed over the three year period from both farms. No *Salmonella* Typhimurium was detected from any samples tested on either farms, but *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* were detected at several points on the one farm. Overwhelming evidence suggests that livestock on the farm that also use the irrigation water source dam as a watering hole, is the source of the *E. coli* O157:H7 contamination. This was shown through the positive *E. coli* O157:H7 contaminated water sources sampled further down the irrigation scheme as well as within the packhouse. It was also found in this study that the facility and personal hygiene was not being effectively managed due to the presence of indicator organisms *S. aureus* on packhouse fruit and in the packhouse on various contact surfaces, including hands of workers. To prevent contamination, it is therefore important to ensure that water sources are potable and the food safety management system is functioning effectively.

1. Introduction

Escherichia coli has been detected from surfaces of plums (Abdelnoor *et al.*, 1983), strawberries (CDC, 2006a) and table grapes (CSPI, 2008), *Salmonella* spp. have been detected from the surface of mango (Sivapalasingam *et al.* 2003), cantaloupe (CSPI, 2008), plums (CDC, 2006a), strawberries (CDC, 2006a; CSPI, 2008) and table grapes (CSPI, 2008) and *Staphylococcus aureus* was detected on lemons (CSPI, 2008) and cantaloupes (CSPI, 2008). Pathogen presence on surfaces of fruit and vegetables does not necessarily lead to contamination of a processed product nor does it indicate a food safety risk at the end of the supply chain to the final consumer. *Escherichia coli*, *Salmonella* spp. and *S. aureus* have been reported to be found in processed products, namely, apple juice (Besser *et al.*, 1993, CDC, 1975), orange juice (CDC, 2005) and banana pudding (CDC, 2004), respectively. Detection of these pathogens is an indication that a foodborne outbreak is possible and that the food safety management system is not properly controlled. Foodborne outbreaks are sporadic and have been reported to have caused up to 5 000 deaths in the USA per year (Gerner-Smidt, 2006). The number of outbreaks that occur per year is considered underreported (Mead *et al.*, 1999).

Escherichia coli O157:H7, *L. monocytogenes* and *Salmonella* spp. have been reported to have caused foodborne disease outbreaks on fresh produce. Two documented cases of *L. monocytogenes* outbreaks were associated with fresh produce in the USA. In 1986 and 1983, *L. monocytogenes* was implicated to cause an outbreak following consumption of contaminated lettuce or celery and coleslaw, respectively (Ho *et al.,* 1986; Schlech *et al.,* 1983). A large disease outbreak of *E. coli* O157:H7 occurred in September 2006 and of *E. coli* O104:H4 in June 2011 in the USA and European Union (EU), respectively (CDC, 2006b; EFSA, 2011; WHO, 2011). The outbreak in the EU during 2011 was linked to the consumption of contaminated bean sprouts and the outbreak in the USA during 2006 was linked to the consumption of contaminated bagged spinach (CDC 2006b; EFSA, 2011; WHO, 2011). The most recent outbreak of *Salmonella* occurred during the period of April to August 2008 and was linked to the consumption of raw tomatoes, jalapeño and cilantro (CDC, 2008a). A second outbreak in 2008 was associated with cantaloupe (CDC 2008b). During March 2011, the CDC had connected 12 cases of *Salmonella* poisoning with the consumption of imported cantaloupes, by June 2011 a total of 20 people had fallen sick from this outbreak of *Salmonella* Panama (CDC, 2011).

The increase in fresh produce associated outbreaks lead to a number of investigations into the presence of foodborne human pathogens on fresh produce and within the growing

environment. These studies included a variety of fresh produce commodities like cantaloupe, oranges, parsley and various fruit and vegetables (Duffy *et al.*, 2005; Johnston *et al.*, 2005; Mukherjee *et al*., 2004; Riordan *et al*., 2001). Different organisms were also investigated, for example Riordan *et al.* (2001) investigated coliforms and faecal coliforms, Duffy *et al.* (2005), Johnston *et al.* (2005), Mukherjee *et al*. (2004) and Riordan *et al*. (2001) all investigated the presence of *E. coli* and *Salmonella* spp. All authors found varying rates of contamination for all organisms and for commodities and the growing environment, with a variation between 39.4% and no detection.

A number of factors lead to produce contamination, potentially leading to outbreaks. Factors include contamination through contact with contaminated soil, improperly composted manure used for compost, contaminated harvesting equipment not cleaned adequately or regularly enough and food handlers not practicing proper personal hygiene (Beuchat, 2002; Beuchat and Ryu, 1997; Hillborn *et al.*, 1999). Fresh produce that is exported from developing countries is thought to pose a greater risk to consumers due to perceived poorer personal and facility hygiene conditions and the use of potentially contaminated water. Various countries and companies therefore began a drive to improve fruit safety by trying to control production practices by implementing voluntary and regulatory good agricultural practices and food safety standards for producers. Europe is one of South Africa's largest importers of fresh produce and EU retailers require compliance to one or more food safety management systems (Dorling *et al*., 2008 as cited by Jacxsens *et al.*, 2010).

Food safety standards and systems that target zero microbial contamination have been developed specifically for the food processing industries. However, the general philosophy is that zero tolerance is not realistic in a pre-harvest environment. Since microbiological analysis of food is time consuming, the International Commission on Microbiological Specification for Foods stated in 1986 that "*Good Agricultural Practices (GAP) and acceptable hygienic farming practices are more important than microbiological testing of food samples before selling*" (Food Science Australia, 2000).

The aim of this study was to evaluate the potential presence of *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium and *S. aureus* in water, on fruit and contact surfaces and to determine possible contamination points related to the use of irrigation water and postharvest practices over two seasons on two peach-producing farms in the Western Cape and Limpopo Province. Total coliforms, faecal coliforms, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* presence was assessed in water.

The absence or presence of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* within the orchard and packing environments was determined.

2. Materials and Methods

2.1. Farm Selection

2.1.1. Peach Farm 1

A peach (*Prunus persica*) farm was selected in the Limpopo Province of South Africa in a region that produces 16% of local peaches. Other crops cultivated on the farm include citrus and maize while game and livestock farming also takes place on the same farm. The farm is managed according to conventional commercial production practices and the farm is Global GAP certified, through the Perishable Products Export Control Board. The farm's packhouse is located near the orchards on the farm (Figure 4.1 D). The climate of this area is a bushveld climate, with an average annual rainfall of 473 mm and a mean summer temperature of 28.8^{o}C. The selection of the farm was based on the farmer's willingness to allow for sampling, presence of dams and an on-site packhouse (Figure 4.1 A, B and D). The farm was visited for four consecutive seasons, 2006/2007, 2007/2008, 2008/2009 and 2009/2010. The exact dates of the field trips are outlined in Table 4.1. The farm was visited once during the harvesting period in 2006/2007 and it was then deemed necessary to visit the farm twice per season. Three weeks prior to harvesting during the spray period, only water samples were collected and at harvest water, fruit and contact surface samples were collected. During the 2008/2009 season just before harvesting a hailstorm destroyed the crop (November 2008), therefore during the 2009/2010 season the farm was visited again on 4 December 2009, for fruit and contact surface sampling.

2.1.2. Peach Farm 2

A peach farm was selected in the Western Cape Province, which is one of the most important stone fruit producing areas in South Africa (51% of national production). Other crops cultivated on this farm include quinces and prickly pear. The farm is managed according to organic production practices and is certified according to the SGS organic standard by SGS South Africa since 2004. The climate of this area is a Mediterranean climate, with an average annual rainfall of 220 mm and a mean summer temperature of 29^{o}C. The farm selection strategy was the same as for Peach Farm 1. The farm was visited

for three consecutive seasons, 2006/2007, 2007/2008 and 2008/2009. The timing of the field trips was similar to that of Peach Farm 1 and the exact dates are outlined in Table 4.1.

2.2. Water sample selection and sampling

Peach farm 1 has two on-site holding dams that collect water from the Phalala River (Figure 4.1). The Phalala River is a significant watercourse in the Waterberg area. This river's catchment basin is a sub-watershed of the Limpopo River. Water was also collected from the river flowing between Dam 1 and Dam 2 (Table 4.1). Water was collected from the river, Dam 1, Dam 2 and pesticide fill point, twice in one season. Water from the packhouse was collected at the time of harvest (when the packhouse was in operation) (Table 4.1). Water samples (five replicates of 1000 ml for filtration and 100 ml for Colilert-18®) were collected from the dams. The samples were collected as far into the dam as physically possible using a telescopic water sampling arm. Water samples were then transported back to the laboratory in a cooling box, with ice packs and refrigerated (4^{o}C) and processed within 24 hours after collection.

Peach Farm 2 is supplied by natural water sources. The farm has two on-site collection dams, of which only one was sampled due to accessibility. Water collects into a dam from the Langeberg Mountains (Figure 4.2). The farm is enclosed by the Langeberg Mountains to the north-east and lower hills to the south, creating a valley (Figure 4.2). Water samples (five replicates of 1000 ml for filtration and 100 ml for Colilert-18®) were collected from the one dam. The sampling protocol was as outlined for Peach Farm 1. Water was also collected from the plant protection product fill point (spray point), originally sourced from the dam sampled (Figure 4.2 A) (Table 4.1). Colilert-18® tests were not conducted during the 2006/2007 season. Water samples were then transported and processed as outlined for Peach Farm 1.

Table 4.1: A seasonal guide for number of samples collected for pathogen detection on South African peach farms

Farm	Type of field trip	Date and number of samples collected	Season			
			2006/2007	2007/2008	2008/2009	2009/2010
Peach Farm 1 Limpopo conventional	Last spray	Date	NI[x]	29 September 2007	5 November 2008	NI
		Water samples	ND[y]	15	15	ND
	Harvest	Date	04 January 2007	10 October 2007	09 December 2008	04 December 2009
		Water samples	10	25	ND	ND
		Fruit samples	20	30	ND	10
		Contact surface samples	ND	138	ND	141
Peach Farm 2 Western Cape organic	Last spray	Date	NI	29 January 2008	22 February 2009	NI
		Water samples	ND	15	15	ND
	Harvest	Date	22 February 2007	26 February 2008	5 March 2009	NI
		Water samples	10	15	15	ND
		Fruit samples	16	20	ND	ND
		Contact surface samples	30	27	ND	ND

[x]: NI indicates that the field Trip was not included in the series of field trips; [y]: ND indicates that so samples were collected.

Figure 4.1: Google Earth Image of Peach Farm 1, situated in Limpopo Province.

A: Dam 1; B: Dam 2; C: Orchard where samples were collected; D: Indicates the location of the packhouse on the farm.

Figure 4.2: Google Earth Image of Pach Farm 2, situated in the Western Cape.

A: Dam 1; B: Orchard where samples were collected.

2.3. Fruit sample selection and sampling

Peach (cv. Oom Sarel) from Peach Farm 1 and Peach (Kakamas) samples from Peach Farm 2 were randomly collected from five trees from a single orchard block (Figure 4.1 C), at four points per tree (Table 4.1). The four points per tree, one sample each from the east, west, inner section of the tree and then on the drip line of the tree, were to determine if the position on the tree would influence the contamination of the fruit. Five fruit samples of three fruit were randomly collected before and after packing only for Peach Farm 1 (Table 4.1). Fruit samples were then transported back to the laboratory in a cooling box, with an ice pack as soon as possible to ensure that the samples remained cool and subsequently stored at refrigeration temperature and processed within 48 hours after collection.

2.4. Contact surface sample strategy and sampling

Transport swabs with Amies medium (Lasec, Johannesburg, South Africa) were wetted in the transport medium and then used to sample a 25 cm^2 area of all contact surfaces in the orchard including hands of pickers and pickers' crates from Peach Farm 1 and Peach Farm 2, the only difference being the numbers of samples collected per sample as is evident in Table 4.1. On Peach Farm 1 the crates were cleaned prior to return to the orchard after off-loading of fruit (Table 4.1). In Peach Farm 1's packhouse the hands of sorters and packers were sampled, as well as floors and/or walls in the packhouse (floors) and cold room (floors and walls) (Table 4.1). The bathroom and wash station taps and the processing line (sort- and pack-line) surfaces were also sampled (Table 4.1). All packhouse equipment was recorded as being cleaned daily. Swab samples were then transported back to the laboratory in a cooler box, with an ice pack as soon as possible to ensure that the samples remained cool and subsequently stored at refrigeration temperature and processed within one week after collection.

2.5. Bacterial Strains

American Type Culture Collection (ATCC, Manassas, United States of America) cultures, maintained as outlined in section 2.1 of Chapter 3 were used as positive controls for all experiments for the DNA extraction as well as for the Polymerase chain reaction (PCR).

2.6. Sample processing

Water samples collected (1000ml) were filtered using a Millipore filtration system through a 0.45µm nitrocellulose filter (Sartorius, Johannesburg). The nitrocellulose filter was transferred into 9ml TSB (Merck, Johannesburg), shake incubated (100rpm) at 37^{o}C for 48 hours followed by DNA extraction and PCR (as outlined in Chapter 3, section 2.3 and 2.4).

Water samples collected (100ml) were processed for Colilert-18® (Dehteq, Johannesburg, SA) analysis as per manufacture's instructions. Results were recorded and the most probable number (MPN) of *E. coli* and coliforms were determined using the MPN generator software program version 3.2 (Idexx, USA).

Fruit samples collected (three fruit per sample) were washed in 500ml quarter strength Ringer's solution (Merck) amended with 0.02% Tween-80 (Sigma, Johannesburg) in an ultrasonic bath for 30 seconds. The inoculated Ringer's solution sample was subsequently filtered through a 0.45µm nitrocellulose filter which was subsequently used to inoculated 9ml TSB and incubated and prepared for DNA extraction and PCR (as outlined in Chapter 3, section 2.3 and 2.4).

A single swab sample was aseptically transferred to TSB (9ml) prior to PCR detection. The TSB was shake incubated (100rpm) at 37^{o}C for 48 hours, followed by a DNA extraction and PCR (as outlined in Chapter 3, section 2.3 and 2.4).

2.7. Statistical Analysis

A pathogen contamination rate was calculated for each season which was a ratio of the number of positive pathogens detected to the number of total samples collected for the specific season. An overall contamination rate was also calculated to be a ratio of the number of positive detections to the total number of samples collected on the farm throughout the study. These contamination rates are expressed as a percentage.

Statistical analysis was performed on the most probable Colilert 18® numbers for the water samples tested. Statistical analysis on the repeated water sample data was done using GenStat for Windows Discovery Edition 2 Software (VSN International Ltd., Lawes Agricultural Trust, UK). A one-way analysis of variance (no blocking) was used to determine the significant differences between times of sampling as well as between sampling location.

Means were analysed using Fischer's protected t-test least significant difference at a 1% level of significance.

3. Results

3.1. Peach Farm 1

The precipitation on farm in the Limpopo Province was 24.4mm, 11.7mm, 43mm, 5.6mm, 8mm, 29.8mm ten days prior to the various sampling periods, respectively (South African Weather Services, 2012). The rainfall was the highest ten days prior to the sampling period, when (South African Weather Services, 2012). During 2007/2008, 43mm of precipitation was recorded between the spray and harvest sampling period (South African Weather Services, 2012). During 2008/2009 rainfall was recorded as 89.6mm (South African Weather Services, 2012).

Coliform titres were significantly lower during the 2007/2008 spray period than during the harvest period at all three locations (Dam 1: *P=0.0019;* Dam 2: *P<0.0001*; River: *P<0.0001*) and *E. coli* titres were lower during the time of harvest than during the time of spray for Dam 1 (*P<0.0001*), Dam 2 (*P<0.0001*) and the river (*P<0.0001*) sampled during 2007/2008 season (Table 4.2). Coliforms titres of water collected from Dam 1 during 2008/2009 season followed the same trend as during 2007/2008 where counts were higher at harvest than at the time of spraying (*P=0.0001*). In this case the other locations were not significantly different (Dam 2: *P=0.0634*; river: *P=0.2080*) (Table 4.2). *Escherichia coli* titres were lower in water collected during harvest than at spray, for Dam 1 (*P=0.003*), Dam 2 (*P=0.0167*) and the river (*P=0.0042*) during the 2008/2009 season, the same trend was seen during the 2007/2008 season (Table 4.2).

Coliform titres during the 2007/2008 season in water collected from Dam 1 were significantly higher than that in Dam 2 and the River (*P<0.0001*), sampled during 2008/2009. The coliforms enumerated from Dam 1 and Dam 2 were lower than that of the river sampled (*P=0.0004*) (Table 4.2). *Escherichia coli)* were present in water collected from Dam 1 in significantly higher numbers than that in Dam 2, which had higher *E. coli* titres than the river during the 2007/2008 spray season (*P<0.0001*) (Table 4.2). During the 2008/2009 spray season the titres of *E. coli* present in water collected from various locations followed the same trend as the coliforms during the same season (*P=0.0002*) (Table 4.2). Coliforms detected from water collected from Dam 1, Dam 2 and the river during the 2007/2008 harvest season were higher than that of the spray point which was in turn higher than that of the

wash station water (*P<0.0001*) (Table 4.2). The same trend was observed during the 2008/2009 harvest season (*P<0.0001*) (Table 4.3). During the 2007/2008 harvest season *E. coli* titres in water collected from Dam 1 and 2 were significantly higher than in water collected from the river, spray point and wash station (*P<0.0001*) (Table 4.2). Water collected from Dam 1, during the 2008/2009 harvest season, had the lowest level of *E. coli*, followed by Dam 2 and then by the wash station and finally by the river which was significantly the highest of all locations (*P<0.0001*) (Table 4.2).

Coliforms in the water collected during the spray season from Dam 1 were not significantly different between the two years (*P=0.4909*), but coliforms enumerated from Dam 2 (*P=0.*0042) and the river (*P<0.0001*) were significantly higher during 2008/2009 than during the 2007/2008 spray season (Table 4.2). The *E. coli* titre of Dam 1 (*P<0.0001*) and Dam 2 (*P=0.0001*) were significantly higher in the 2007/2008 than in the 2008/2009 spray season, but the river had the reverse trend (*P=0.0034*) (Table 4.2). Coliforms enumerated from water collected during the harvest season of 2007/2008 and 2008/2009 were not significantly different (Dam 1: *P=0.3739*; Dam 2: *P=0.4766*; river: *P=0.2080*), except at the wash station (*P=0.0016*) (Table 4.2). During the 2008/2009 harvest period the wash station had significantly more coliforms than during 2007/2008 (*P=0.0016*) (Table 4.2). The *E. coli* titres in water collected during the 2007/2008 harvest season were not significantly different to those enumerated during the 2008/2009 season when comparing between the two years at Dam 2 (*P=0.*9466) and the wash station (*P=0.0772*) (Table 4.2). Water collected from Dam 1 had *E. coli* titres that were significantly higher during 2007/2008 than 2008/2009 harvest season (*P=0.0038*), and the *E. coli* titres of water collected from the river during 2008/2009 harvest season were significantly higher than the 2007/2008 season (*P<0.0001*) (Table 4.2).

No *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* were detected from the 65 water samples collected on Peach Farm 1 (Table 4.3). During the harvest period of the 2007/2008 season, five samples tested positive for the presence of *E. coli* O157:H7 (Table 4.3). Two river and three Dam 1 samples were contaminated with *E. coli* O157:H7 (Table 4.3). The five *E. coli* O157:H7 samples contaminated resulted in a 20% water contamination rate for *E. coli* O157:H7 during the 2007/2008 harvest period and a 7.7% *E. coli* O157:H7 contamination rate overall for Peach Farm 1 (Table 4.3).

No *Salmonella* Typhimurium was detected on the 50 fruit samples collected from Peach Farm 1. *Escherichia coli* O157:H7 and *S. aureus* were detected on one fruit sample after packing in the packhouse during the 2007/2008 season, resulting in a 3.33% fruit contamination for 2007/2008 (Table 4.4). *Listeria monocytogenes* was detected on one fruit

Table 4.2: Peach Farm 1's Colilert-18® coliform and *Escherichia coli* results of water samples expressed in most probable number per 100ml

Sampling Period	Sampling Point	Season											
		2007/2008			2008/2009			2007/2008			2008/2009		
		Coliforms						*Escherichia coli*					
		MPN[x]			MPN		*P* value	MPN			MPN		*P* value
Water samples collected at last spray date	Dam 1	1168.70	**B** a *A*		1259.20	**B** b *A*	0.4909	202.33	**A** a *A*		12.54	**A** b *B*	<0.0001
	Dam 2	134.83	**B** b *B*		1301.20	**A** b *A*	0.0042	160.70	**A** b *A*		45.26	**A** b *B*	0.0001
	River	186.47	**B** b *B*		2419.60	**A** a *A*	<0.0001	25.43	**A** c *B*		161.80	**A** a *A*	0.0034
	***P* value**[y]	<0.0001			0.0004			<0.0001			0.0002		
Water samples collected at harvest	Dam 1	2282.30	**A** a *A*		2419.60	**A** a *A*	0.3739	19.94	**B** a *A*		2.10	**B** d *B*	0.0038
	Dam 2	2332.90	**A** a *A*		2159.60	**A** a *A*	0.4766	23.90	**B** a *A*		24.12	**B** b *A*	0.9466
	River	2419.60	**A** a *A*		2159.60	**A** a *A*	0.208	7.90	**B** b *B*		30.30	**B** a *A*	<0.0001
	Spray point	435.60	b		ND[z]		-	4.38	b		ND		-
	Wash station	57.30	c *B*		410.20	a *A*	0.0016	3.12	b *A*		6.56	c *B*	0.0772
	***P* value**	<0.0001			<0.0001			<0.0001			<0.0001		

[x]: Most probable number per 100 ml. [y]: *P* value is significant if the value is less than 0.05; [z]: ND indicates the location was not sampled during the specified season. Most probable values followed by the same **CAPITAL LETTER** indicate no significant difference between the spray and harvest period within a specific year. Values followed by the same lowercase letter indicate no significant difference at the different locations within a sampling period within one year. Values followed by the same *ITALICISED CAPITAL LETTER* indicate no significant difference at a location between 2007/2008 and 2008/2009.

sample within the orchard during the 2006/2007 season, resulting in a 5% *L. monocytogenes* fruit contamination rate (Table 4.4). Overall, a 2% fruit contamination rate for *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* was determined.

No *Salmonella* Typhimurium was detected from the 279 contact surface samples collected from Peach Farm 1. During the 2007/2008 season, 13% of the contact surfaces were contaminated with *E. coli* O157:H7, with ten samples contaminated with *E. coli* O157:H7 from the packhouse floors and four from pickers' crates and only one detected from the wash station tap, sortline, packer's hand and the cold room wall (Table 4.4). During the 2009/2010 season, the *E. coli* O157:H7 contamination rate for contact surfaces was significantly reduced to only 0.71% with only one sample from the packhouse floors detected (Table 4.4). Overall, Peach Farm 1 had an *E. coli* O157:H7 surface contamination rate of 6.8% (Table 4.4).

One contact surface sample and one picker's hand during the 2007/2008 season demonstrated the presence of *L. monocytogenes* resulting in a 0.7% surface contamination rate (Table 4.4). During the 2009/2010 season, only one contact surface sample, and a cold room floor sample were contaminated with *L. monocytogenes* resulting in a 0.71% *L. monocytogenes* surface contamination rate (Table 4.4). The overall *L. monocytogenes* contamination rate for Peach Farm 1 was 0.72% (Table 4.4).

Staphylococcus aureus was detected from five of the 138 contact surface samples collected during 2007/2008 season, resulting in a 3.6% surface contamination rate for *S. aureus* (Table 4.4). Three pickers' hands, one wash station tap and one cold room floor sample was contaminated with *S. aureus* (Table 4.4). Only one sample demonstrated the presence of *S. aureus* in the 2009/2010 season, from a packer's hand (Table 4.4). This resulted in a 0.71% *S. aureus* contamination rate for 2009/2010 season. Overall the *S. aureus* surface contamination rate for Peach Farm 1 was 2.15%.

3.2. Peach Farm 2

Peach Farm 2 is situated in a winter rainfall area, ten days prior to all sampling periods no precipitation was recorded, except during the 2007/2008 spray period where 0.5mm fell (South African Weather Services, 2012). Between the two sampling periods in 2007/2008 season 17.1mm rainfall was recorded and 43mm in 2008/2009 (South African Weather Services, 2012).

During 2007/2008, coliforms and *E. coli* in water collected from the dam (Coliforms: *P=0.0352, E. coli*: *P= 0.0002*) and the pesticide spray point (Coliforms: *P=0.0102*; *E. coli*: *P= 0.0135*) were significantly higher during the time of harvest, but coliforms and *E. coli* enumerated from water collected from the dam inlet (Coliforms: *P=0.0042; E. coli*: *P=0.0121*) were higher during the spray season (Table 4.5). During the 2008/2009 season, water collected from the dam (*P=0.5131*), dam inlet (*P=0.3731*) and the spray point (*P=0.6055*) were not significantly different between the spray and harvest period (Table 4.5). *Escherichia coli* enumerated from water collected from the dam inlet and pesticide spray point (*P=0.231*) during the 2008/2009 season showed no significant difference between spray and harvest period (Table 4.5). During 2008/2009 the *E. coli* titres of water collected from the dam (*P=0.0466*) were significantly higher during the harvest period than in the spray period (Table 4.5).

Coliforms enumerated during the 2007/2008 spray season were highest in water collected from the dam inlet, then the dam (which was significantly lower than the dam inlet) and then the spray point (which was significantly lower than the dam) (*P<0.0001*) (Table 4.5). During the 2008/2009 spray and harvest season it was demonstrated that the dam and spray water contained coliform titres that were not significantly different, but the dam inlet contained significantly lower titres (*P<0.0001*) (Table 4.5). The dam contained the highest number of coliforms during 2007/2008 harvest period, followed by the dam inlet and then the pesticide spray point, these were all significantly different to one another (*P=0.0002*) (Table 4.5). During the 2007/2008 spray season the pesticide spray point contained significantly more *E. coli* than the dam and dam inlet (*P=0.0006*) (Table 4.5). There was no significant difference in all locations during 2008/2009 spray season (*P=0.4999*) (Table 4.5). During 2007/2008 harvest season the water collected from the dam inlet had significantly higher *E. coli* titres than the dam, which had significantly higher *E. coli* titres than the pesticide spray point (*P<0.0001*) (Table 4.5). The dam and spray point *E. coli* titres during the 2008/2009 harvest period were not significantly different, neither were the dam inlet and the spray point (*P=0.0396*) (Table 4.5).

No *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* were detected in the 55 water samples, nor on 36 fruit samples (Table 4.6) collected on Peach Farm 2, resulting in no contamination.

Table 4.3: The number of samples tested positive for a specific organism using the multiplex PCR in water samples collected on Peach Farm 1

Sample location	*Escherichia coli* O157:H7			*Listeria monocytogenes*			*Staphylococcus aureus*		
	2006/2007	2007/2008		2006/2007	2007/2008		2006/2007	2007/2008	
	Harvest	Spray	Harvest	Harvest	Spray	Harvest	Harvest	Spray	Harvest
River (n[x]=15)	0[y]	0	2	0	0	0	0	0	0
Dam 1 (n=5)	ND[z]	ND	3	ND	ND	0	ND	ND	0
Dam 2 (n=15)	0	0	0	0	0	0	0	0	0
Spray point (n=10)	ND	0	0	ND	0	0	ND	0	0
Wash station (n=5)	ND	ND	0	ND	ND	0	ND	ND	0

[x]: Indicates the total number of samples collected from each sample location; [y]: Indicates the number of samples that tested positive for a specific organism; [z]: ND indicates the location was not sampled during the specified season.

Table 4.4: The number of samples tested positive for a specific organism on Peach Farm 1 by using the multiplex PCR on samples taken at harvest

Sample Location	*Escherichia coli* O157:H7			*Listeria monocytogenes*			*Staphylococcus aureus*		
	2006/2007[a]	2007/2008[b]	2009/2010[c]	2006/2007	2007/2008	2009/2010	2006/2007	2007/2008	2009/2010
Trees (n[x]=40)	0[y]	0	ND	1	0	ND	0	0	ND
Fruit before or after packing (n=20)	ND[z]	1	0	ND	0	0	ND	1	0
Pickers' hands (n=20)	ND	0	0	ND	1	0	ND	3	0
Pickers' crates (n=26)	ND	4	0	ND	0	0	ND	0	0
Bathroom taps (n=20)	ND	0	0	ND	0	0	ND	0	0
Wash station taps (n=12)	ND	1	0	ND	0	0	ND	1	0
Sortline (n=18)	ND	1	0	ND	0	0	ND	0	0
Sorter's hands (n=17)	ND	0	0	ND	0	0	ND	0	0
Packline (n=20)	ND	0	0	ND	0	0	ND	0	0
Packer's hands (n=20)	ND	1	0	ND	0	0	ND	0	1
Packhouse floors (n=60)	ND	10	0	ND	0	0	ND	0	0
Cold room floors (n=30)	ND	0	1	ND	0	1	ND	1	0
Cold room walls (n=30)	ND	1	0	ND	0	0	ND	0	0
Total samples positive	**0**	**20 (11.9%)**	**1 (0.65%)**	**1 (5%)**	**1 (0.6%)**	**1 (0.65%)**	**0**	**6 (3.6%)**	**1 (0.65%)**

[x]: Indicates the total number of samples collected from each sample location; [y]: Indicates the number of samples that tested positive for a specific organism; [z]: ND indicates the location was not sampled during the specified season; [a]: During 2006/2007 season 20 samples were collected; [b]: During 2007/2008 season 168 samples were collected; [c]: During 2008/2009 season 155 samples were collected.

Table 4.5: Peach Farm 2's Colilert-18® coliform and *Escherichia coli* results of water samples expressed in most probable number per 100ml

Sampling Period	Sampling Point	Season					
		2007/2008	2008/2009		2007/2008	2008/2009	
		Coliforms			*Escherichia coli*		
		MPN[x]	MPN	*P* value	MPN	MPN	*P* value
Water samples at last spray	Dam	1311.80 **B** b *A*	117.14 **A** a *B*	0.0004	0.60 **B** b *A*	3.08 **B** a *A*	0.0815
	Dam inlet	2419.60 **A** a *A*	16.64 **A** b *B*	<0.0001	3.28 **A** b *A*	1.62 **A** a *A*	0.2943
	Spray Point	87.64 **B** c *B*	137.98 **A** a *A*	0.0266	10.48 **B** a *A*	2.86 **A** a *B*	0.0011
	***P* value**[y]	<0.0001	<0.0001		0.0006	0.4999	
Water samples at harvest	Dam	1994.40 **A** a *A*	127.58 **A** a *B*	0.002	6.50 **A** b *A*	5.02 **A** a *A*	0.285
	Dam inlet	1385.30 **B** b *A*	19.20 **A** b *B*	0.0015	1.00 **B** a *A*	1.62 **B** b *A*	0.3682
	Spray Point	198.00 **A** c *A*	151.70 **A** a *A*	0.1866	18.48 **A** c *A*	3.70 **A** ab *B*	0.0014
	***P* value**	0.0002	<0.0001		<0.0001	0.0396	

[x]: Most probable number per 100 ml. [y]: *P* value is significant if the value is less than 0.05. Most probable values followed by the same **CAPITAL LETTER** indicate no significant difference between the spray and harvest period within a specific year. Values followed by the same lowercase letter indicate no significant difference at the different locations within a sampling period within one year. Values followed by the same *ITALICISED CAPITAL LETTER* indicate no significant difference at a location between 2007/2008 and 2008/2009.

No *Salmonella* Typhimurium, *E. coli* O157:H7 or *L. monocytogenes* was detected on contact surface samples collected from Peach Farm 2, resulting in a 0% contamination rate. Only *S. aureus* was detected on one Picker's hand, resulting in a 3.33% *S. aureus* surface contamination rate and an overall 1.75% surface contamination rate from Peach Farm 2 (Table 4.6).

Table 4.6: The number of samples tested positive for a specific organism on Peach Farm 2 by using the multiplex PCR from samples taken at harvest

Sample location	*Escherichia coli* O157:H7		*Listeria monocytogenes*		*Staphylococcus aureus*	
	2006/2007[a]	2007/2008[b]	2006/2007	2007/2008	2006/2007	2007/2008
Trees (n^x=40)	0^y	0	0	0	0	0
Pickers' hands (n=27)	0	0	0	0	1	0
Pickers' bucket (n=20)	0	0	0	0	0	0
Pickers' crates (n=10)	0	0	0	0	0	0
Total samples positive	**0**	**0**	**0**	**0**	**1 (2%)**	**0**

[x]: Indicates the total number of samples collected from each sample location; [y]: Indicates the number of samples that tested positive for a specific organism; [a]: During 2006/2007 season 50 samples were collected; [b]: During 2007/2008 season 47 samples were collected

4. Discussion

The safety of fresh produce in the growing and packaging environment has become a minimum requirement to ensure compliance with minimum food safety standards. It is therefore necessary to determine the level of risk associated with foodborne disease outbreaks. In this study, *Salmonella* Typhimurium was not detected in water, fruit or contact surfaces on both peach farms sampled. This study thus confirms the absence of *Salmonella* Typhimurium in the peach on-farm production and packaging systems. McMahon and Wilson (2001) and Johannessen *et al.* (2002) did also not detect the presence of *Salmonella* species from fresh produce or water sampled. It is possible that other strains of *Salmonella* species could have been present as was the case in studies conducted with water (Duffy *et al.* 2005) and fresh produce (Mukherjee *et al.*, 2004).

Cooley *et al.* (2007) determined a 12.16% presence of *E. coli* O157:H7 in environmental samples (water, soil and dust) for the two farms they investigated. These figures corresponded with our findings of 20% prevalence of *E. coli* O157:H7 in water samples for the 2007/2008 season. Over the two year period of this study a 7.7% water contamination rate for one of the farms was recorded. Surface water quality is difficult to control due to

possible contamination by livestock and human activity (Kirby *et al.*, 2003; Odumeru *et al*., 2004). The presence of *E. coli* O157:H7 in the one dam in this study indicates that livestock could have contaminated the water on this farm. It was interesting to note that only this farm had livestock (cattle) using this water source, which correlates with the presence of *E. coli* O157:H7 according to findings in another study by Hagedorn *et al.* (1999). On the contaminated farm, the one dam sampled is also being used as a watering hole for livestock which was found to be contaminated with *E. coli* O157:H7. It was noted at the time of sampling that the faeces accumulated on the banks of the dam were mixed with the water as water levels increased due to rain. The water from this dam is used for irrigation purposes on the farm and gets distributed to various points on the farm via the river, which was also found to contain *E. coli* O157:H7 over the season evaluated. The dam also reflected the increase in coliform titres over the season, further confirming that livestock faeces is the source of contamination. Water samples that were studied by Cooley *et al.* (2007) demonstrated that the generic *E. coli* counts increased five-fold during the flooding season, due to contaminants mixed with water as water levels increased. On this same farm where the *E. coli* O157:H7 was detected in the dam, the same pathogen was also detected from river water samples. This pathogen was also detected from peach fruit collected from the packhouse. The possibility that the original source of contamination can be linked to the contaminated water source is thus highly likely and should be confirmed by future typing studies. It was however significant that the same pathogen was detected from fruit, water and within the packhouse, in contrast to the other farm that was considered free of *E. coli* O157:H7 using the current sampling protocol and test methods.

Riordan *et al* (2001) detected no *E. coli* O157:H7 out of 36 water samples (10ml each) and 230 fruit samples collected from the 14 farms under investigation around the USA. Selma *et al*. (2007) detected no *E. coli* O157:H7 from the three farms investigated in Spain, the authors collected three water samples (100ml was processed) and ten produce samples per visit and visited the farms four times within the season. In contrast however, Izumi *et al.* (2008) did detect *E. coli* O157:H7 in water collected from the Japanese persimmon farm, sampled, these authors collected only 21 water samples over a seven month period, however no *E. coli* O157:H7 were detected on the fruit peel or flesh out of 144 fruit samples. Even though sample size and amount per sample for the previously mentioned studies are similar, only one detected the presence of *E. coli* O157:H7 in water. This current study used molecular methods to detect and confirm the presence of *E. coli* O157:H7 from the two farms sampled with a total of 50 (1000 ml) samples analysed and 60 fruit tested. The number of samples tested in the current study and methods are comparable with similar studies.

During this study *E. coli* O157:H7 was also detected on peach crates, sortline and a packer's hands all of which could have been potential sources of fruit contamination. All contact surfaces on this farm are washed using filtered water from one of the dams or with water from underground water sources. At the time of sampling the water used for cleaning purposes tested negative for *E. coli* O157:H7. Duffy *et al.* (2005) also detected *E. coli* on contact surfaces of the boxing ramps, conveyer belts, plastic bags and bins, receiving hopper, trailers for transport and the unloading ramp. This resulted in a 9.3% *E. coli* environmental contamination rate. This data reflects a similar pattern as found in our study on the one farm that had *E. coli* O157:H7. Packhouse and cold room floors as well as cold room walls were also contaminated with *E. coli* O157:H7 on this farm. Eighty percent of *E. coli* O157:H7 detected from samples collected from packhouse floors were found to be at the entrance to the packhouse. The likelihood that the *E. coli* O157:H7 was carried into the packhouse on the soles of workers shoes as they enter the packhouse was considered as a possible entry route particularly since Egyptian geese droppings were noticed in and around the packhouse and near workers toilet facilities at the time of sampling. A previous study conducted found that after a new pair of shoes was worn for two weeks, coliforms and *E. coli* were detected on the outside of the shoes (Cleaning Industry Research Institute Staff, 2011). This study also demonstrated the transfer of bacteria from the source of origin to the participant's households. Future research should focus on preventative measures, behavioural correction and disinfectant efficacy, to avoid the contamination of the packhouse floors because it can potentially lead to fruit contamination.

No *L. monocytogenes* was detected in water samples collected over a two year period in this study which is in contrast with Selma *et al.* (2007) who found the presence of *Listeria* spp. in creek and residual irrigation water. Selma *et al.* (2007), as outlined previously had fewer water samples when compared to the current study. The current study indicated the presence of *L. monocytogenes* on fruit in the orchard. In Norway, Johannessen *et al.* (2002) detected *L. monocytogenes* using traditional cultural methods with no molecular confirmation on three out of 859 fresh produce samples collected. Pingulkar *et al.* (2001) isolated *L. monocytogenes* from 14 of the 80 washed fresh produce collected locally in India, isolates were identified to species level with traditional methods. McMahon and Wilson (2001) did not detect the presence of *Listeria* spp. from 86 organic vegetables from Northern Ireland using traditional cultural techniques with biochemical tests as confirmation. In the current study *L. monocytogenes* was also detected from picker's hand and cold room floor samples. The continuous detection of *L. monocytogenes* throughout the production environment is consequential evidence that there is an on-farm source of contamination. Future research

should determine the source of the *L. monocytogenes* contamination and compare traditional with molecular detection methods.

In this study, the presence of *S. aureus* was used as an indicator of poor personal hygiene and inadequate facility sanitation. The significant detection of *S. aureus* from a fruit and a picker's and packer's hand and the wash station tap is evidence that the personal and facility hygiene does not comply with minimal food safety management standards. *Staphylococcus aureus* was not detected on fruit that were harvested from low hanging branches of the trees, therefore the contamination of fruit took place through human handling. Johannessen *et al*. (2002) detected that 35 of 156 mushrooms and 26 of 173 strawberries from Norway were contaminated with *S. aureus* using traditional methods and confirmation was done by biochemical tests. This exceeds the contamination rate in this current study. Another author that detected high prevalence of *S. aureus* on fresh produce was Viswanathan and Kaur (2001), who did a survey of fresh produce purchased from street vendors in India. These authors determined that 70 of the 120 fresh produce samples, with 14 isolated from 23 fruit samples collected tested positive for the presence of *S. aureus* using traditional culture medium techniques. The high contamination rate found in the two studies, Johannessen *et al*. (2002) and Viswanathan and Kaur (2001), was due to poor personal and facility hygiene and incorrect storage facilities. Similarly in this study, detection of *S. aureus* can most likely be attributed to poor personal hygiene because hands and the wash station tap handle, was found to be contaminated.

Escherichia coli O157:H7, *L. monocytogenes* and *S. aureus* were detected on fruit as well as on contact surfaces and food handler's hands. Contact with a contaminated surface can lead to cross contamination of the final product prior to leaving the packhouse. All three organisms were detected on either workers hands or on contact surfaces, irrespective of the initial source of contamination. The final product was also found to be contaminated with all three pathogens. Work undertaken as part of the Campden BRI project demonstrated that hands and contact surfaces can transfer *E. coli*, *L. innocua* (a non-pathogenic surrogate of *L. monocytogenes*) or *Staphylococcus* spp. to contact surfaces or fresh produce surfaces (Smith, 2007).

This study revealed that coliforms and *E. coli* were detected in all water sources on the two farms. Traditionally, *E. coli* have been used as indicator system for the presence of animal and human faecal matter. However, potable water should be used at all times in the packhouse (Kirby *et al.*, 2003). Water on the one farm was not found to comply with potable water standards of South Africa (SANS 241:2005). This implication can be considered a

potential risk because hazards are present in the water but these are only perceived risks because only indicator organisms are analysed. The WHO (2006) recommends that *E. coli* levels must not exceed 1000 counts per 100ml for the safe use of wastewater for irrigation. The established values for microbiological parameters on both farms in this study can be considered fit for irrigation purpose according to "*Water Quality Guidelines by the Department of Water Affairs and Forestry* (1996)". Interestingly, the farm studied in this investigation are certified to Global GAP and complies with the national water standards which one [Department of Water Affairs and Forestry (DWAF), 1996] regarding water quality used for irrigation purposes and potable water standards (SANS 241:2005) for use in packhouse wash water. Yet the farm was found to be contaminated with foodborne pathogens indicating the failure of the certification process. Current Global GAP certification standards require a risk assessment approach to be conducted on the farm in order to determine the level of risk and therefore allowing the assurance of effective management of risk. Current water tests required for compliance is only based on indicator systems and do not test for specific pathogens. Future studies should therefore focus on sampling size, frequency of testing and method comparison.

In conclusion, *Salmonella* Typhimurium was not detected on any samples analysed in this study. The other three pathogens, *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* were detected in water, fruit and on contact surfaces and do pose a potential risk to the consumer. A semi-quantitative risk assessment (outlined in Chapter 8) was conducted to determine the real risk to the final consumer. The source of *E. coli* O157:H7 on the one farm was considered to be livestock contaminating the source water. Confirming this link associated with contaminated source water and a food safety hazard on fresh stone fruit should be done in future studies. If this and other potential hazards are present within the production arena and on contact surfaces and hands there exists the potential of fruit contamination within the supply chain.

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Chapter 5

Assessment of possible foodborne pathogen presence on plums and in the production and postharvest environments

Stacey Collignon and Lise Korsten

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Abstract

A plum is a product that is mostly consumed fresh, without cooking or minimal processing and prevention of possible foodborne pathogen product contamination is therefore important. Contamination can happen at various points during production or in the postharvest environment. Due to the ability of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* and *Staphylococcus aureus* to attach to and colonise fruit surfaces, it is important to prevent such contamination by implementing various food safety management control systems and by pre-determining possible contamination points and levels for more effective management strategies in food safety assurance systems. The aim of this study was to determine the prevalence of the above mentioned pathogens in water, on contact surfaces or hands coming into direct contact with the fruit and from fruit within the production and packaging environment. Pathogen prevalence was monitored over two seasons as part of a hazard characterisation study. In this study, no *Salmonella* Typhimurium were detected in water or on fruit or contact surfaces. *Escherichia coli* O157:H7 and *S. aureus* were detected in a water sample collected from one plum farm. *Listeria monocytogenes* and *S. aureus* were detected in water samples from the other plum farm. Fruit samples collected from the one farm where water contamination occurred, were contaminated with *E. coli* O157:H7 and *S. aureus;* however no pathogens were detected from fruit collected from the other farm. Contact surface samples collected from both farms were contaminated with *E. coli* O157:H7 and *L. monocytogenes* but contact surfaces from only one farm demonstrated *S. aureus* contamination. The low prevalence of foodborne pathogens on fruit entering the supply chain means that the number of fruit posing a potential possible risk to consumers is reduced. The presence of some of these pathogens on contact surfaces indicates that the food safety management system needs to be improved to reduce the level of risk.

1. Introduction

Produce food safety and hygiene from production, packing through to transportation is of utmost importance due to increased demands and changing consumption patterns of fresh produce. More wide spread distribution of food products, new retailer requirements and consumer awareness of food safety prevalence now requires more effective management of the food safety assurance systems. A lack of knowledge of the microbial hazards associated with poor personal hygiene and inadequate facility sanitation can lead to an increased risk of product contamination (De Roever, 1998). Prevention of contamination of fresh produce with foodborne pathogens is therefore of importance since fruit are mostly consumed fresh and to a lesser extent minimally processed or processed.

Salmonella enterica serovars and *E. coli* O157:H7 are two known bacterial pathogenic species most often implicated in foodborne disease outbreaks (Tauxe *et al.*, 1997). Therefore, a number of previous studies have focused on the presence of *E. coli* and *Salmonella* spp. on fresh produce as well as in the production environment (Duffy *et al.*, 2005; Johnston *et al.*, 2005; Mukherjee *et al*., 2004; Riordan *et al*., 2001). Duffy *et al.* (2005) studied the presence of *E. coli* O157:H7 and *Salmonella* spp. on cantaloupe, orange and parsley within the fresh produce growing environment. It was determined that 39.4% of water samples, 12.3% of production equipment sampled and 7.3% of fruit samples were contaminated with *E. coli*. *Salmonella* spp. were isolated from 4.38% of water samples, 2.1% of production equipment sampled and 0.5% of fruit samples (Duffy *et al.*, 2005). In another study organic and conventional fresh produce farms were compared and a large range of fruit and vegetables were screened for the prevalence of *Salmonella* spp., *E. coli* and *E. coli* O157:H7 (Mukherjee *et al*., 2004). Mukherjee *et al.* (2004) found that 1.6% of produce from conventional farms and 9.7% of produce originating from the organic farms were contaminated with *E. coli*. No *E. coli* O157:H7 was detected on the produce screened from either conventional or organic farms. *Salmonella* spp. was only detected from one organic lettuce and one organic green pepper sample (Mukherjee *et al*., 2004). Johnston *et al.* (2005) determined that *Salmonella* spp. was present on 0.7% of fresh produce and *L. monocytogenes* and *E. coli* O157:H7 was not detected on produce from production to post-harvest handling. Similarly, no *E. coli* O157:H7 was detected from fruit samples evaluated by Riordan *et al.* (2001). According the FAO/WHO (2008) fruit are considered a low risk product and no cases of foodborne pathogen presence or disease outbreaks have been recorded on plums (*Prunus domesticus*).

Escherichia coli O157:H7 and *Salmonella* spp. are important organisms in the safety assurance framework of fresh produce (Riordan *et al.*, 2001). The presence of foodborne pathogens on fruit after harvest can be a result from poor on-farm orchard and packhouse management practices (Riordan *et al.,* 2001). Fresh produce can become contaminated at any step through the supply chain, from production to retail sale. The production environment is therefore the first point where fresh produce could become contaminated through contact with contaminated irrigation water, soil, compost, harvesting methods, harvesting equipment and food handlers (Beuchat, 2002; Beuchat and Ryu, 1997; Hillborn *et al.*, 1999). *Listeria monocytogenes* has been implicated in a number of outbreaks and Prazak *et al.* (2002) demonstrated that a packhouse is an ideal environment for *Listeria* spp. to proliferate and survive especially on conveyer belts where direct contact with fruit occurs. The absence or presence of *S. aureus* is used as an indicator of poor personal hygiene, because the organism is a natural inhabitant of the human skin (Adams and Moss, 2000; Rediers *et al.*, 2008). Curtis *et al.* (2000) concluded that good hygiene practices are based on usage of potable water and personal hygiene practices that reflect informed behaviour.

Water is an important consideration for the contamination of hands and contact surfaces, because both are cleaned with water. Water to be used in the orchard or packhouse for human consumption or personal hygiene, therefore needs to be potable (Kirby et al., 2003). Water is used more extensively, for irrigation, fertigation and pesticide application within the production environment, and during washing, movement or pesticide application in the postharvest environment all potentially leading to possible contamination (Beuchat and Ryu, 1997; Hillborn *et al.*, 1999; Steele and Odumeru, 2004). Water comes into direct contact with fresh produce at various points in the orchard. It is therefore important to determine the quality of water being used within the orchard, prior to use. By using poor quality, faecally contaminated water for irrigation purposes farmers are compromising the safety of their produce (Mara *et al.* 2007). The microbial quality of water can be assessed by indicator organisms, which reflect the general status and the presence of faecal matter at the source. *Escherichia coli* is the main faecal coliform which is tested for, to reflect water quality. For tree drip irrigation purposes it is recommended that faecal coliforms levels should not exceed 1000 counts per 100 ml (DWAF, 1996; WHO, 2006).

The aim of the study was to evaluate the potential presence of foodborne bacterial pathogens in relation to irrigation water and postharvest processing over two seasons and on two plum-producing farms in the Western Cape and Limpopo Province. Total coliforms, faecal coliforms, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* presence was assessed in water. The absence or presence of *E. coli* O157:H7, *L.*

monocytogenes, *Salmonella* Typhimurium and *S. aureus* within the orchard and packing environments as well as during handling was also determined.

2. Materials and Methods

2.1. Farm selection

2.1.1. Plum Farm 1

A plum farm was selected in the Limpopo Province which is one of the most important stone fruit production areas in South Africa situated in the Northern parts of the country. The farm is managed according to and certified to the organic standard (SGS certification). The farm is also Global GAP certified. The farm's packhouse is located near the orchards, on the same farm (Figure 5.1 D). The climate is typical bushveld with an average annual rainfall of 473 mm and a mean summer temperature of 28.8^{o}C. The selection of the farm was based on the farmer's willingness to provide samples, presence of dams and an on-site packhouse (Figure 5.1 A, B and D). The farm was visited for three consecutive seasons, once during 2006/2007, twice during 2007/2008 and twice during 2008/2009 as is outlined in Table 5.1. The plum picking season for one cultivar in Limpopo Province occurs over a window of two to three weeks. Plums collected from Plum Farm 1 were of the cultivar Flavour King.

2.1.2. Plum Farm 2

A second plum farm was selected in the Western Cape Province, which is one of the most important stone fruit producing areas in South Africa. The farm is managed according to conventional production guidelines. The farm was Euro GAP certified from 2005 (PPECB certification body) and has been Global GAP certified from 2008 to 2011 (PPECB was the certification body from 2008 to 2010 and CMI was the certification body from 2011). The farm's packhouse is also located near the orchards on the farm. The climate of this area is Mediterranean with an average annual rainfall of 50 mm and a mean summer temperature of 26.1^{o}C. The selection of the farm was based on the same strategy as Plum farm 1 (Figure 5.2 A, B and C). The farm was visited for three consecutive seasons at similar intervals to Plum Farm 1, once during 2006/2007, twice during 2007/2008 and twice during 2008/2009, as outlined in Table 5.1. The plum picking season for one cultivar on this farm occurs over a window of two to three weeks. Plums collected from Plum Farm 1 were of the cultivar Laetitia and Songold.

Table 5.1: A seasonal guide for number of samples collected for pathogen detection on South African Plum Farms

Farm	Type of field trip	Date and number of samples collected	Season		
			2006/2007	2007/2008	2008/2009
Farm 1 Limpopo Organic	**Last spray**	**Date**	**NI**[x]	**12 December 2007**	**09 December 2008**
		Water samples	ND[y]	10	15
	Harvest	**Date**	**04 January 2007**	**08 January 2008**	**11 January 2009**
		Water samples	15	25	25
		Fruit samples	30	30	ND
		Contact surface samples	152	151	37
Farm 2 Western Cape Conventional	**Last spray**	**Date**	**NI**	**14 January 2008**	**07 January 2009**
		Water samples	ND	30	30
	Harvest	**Date**	**31 January 2007**	**31 January 2008**	**22 January 2009**
		Water samples	30	35	35
		Fruit samples	46	50	ND
		Contact surface samples	158	165	ND

[x]: NI indicates that the field trip was not included in the series of field trips; [y]: ND indicates that no samples were collected.

Figure 5.1: Google Earth Image of Plum Farm 1, situated in Limpopo Province.

A: Dam 1; B: Dam 2; C: Orchard where samples were collected; D: Indicates the location of the packhouse on the farm, close to the orchards.

Figure 5.2: Google Earth Image of Plum Farm 2, situated in the Western Cape.

A: Dam 1; B: Dam 2; C: Dam 3; D: Orchard where samples were collected.

2.2. Water sample selection and samples collected

Plum Farm 1 has two on-site collection dams that collect water from the Phalala River (Figure 5.1). The Phalala River is a significant watercourse in the Waterberg area. This river's catchment basin is a sub-watershed of the Limpopo River. Water samples (five replicates of 1000 ml for filtration and 100 ml for Colilert-18®) were collected from both dams as far into the dam as physically possible using a telescopic water sampling arm (Table 5.1). Colilert-18® tests were not conducted during the 2006/2007 season. Water was also collected from the area of the dam where water for the pesticide spray car is pumped out (spray point) (sampling was done directly from this site since the pump was not operational at the time of sampling). The packhouse on the farm uses a wet wall system for pre-cooling, the water circulating through the wet wall was collected at the time of harvest (Table 5.1). The water re-circulating through the wet walls is collected into a 100 litre drum. This water was according to the packhouse schedule changed daily, with the addition of Spore Kill (mixed according to manufacturer's instruction). Water samples were immediately transported back to the laboratory in a cooling box, containing ice packs and subsequently stored at refrigeration temperature and processed within 24 hours after collection.

Plum Farm 2 has a number of on-site collection dams, of which only three were sampled. Water is collected into dams from the Lourens River (Figure 5.2). Water samples were from all three dams, as described for Plum Farm 1 (Figure 5.1 and Table 5.1). Water flowing into the water collection system and water after the resident trout farm were also collected (Figure 5.1 C and Table 5.1). Water was also collected from the pesticide fill point (spray point), originally sourced from Dam 2, closest to the orchard block selected for fruit analysis (Figure 5.1 C) (Figure 5.2 D and Table 5.1). A water sample was also collected from the packhouse as described for Plum Farm 1 (Table 5.1). Transportation and processing took place as described for Plum Farm 1.

2.3. Fruit sample strategy and sampling

Plum samples were collected from five trees from a single orchard block for Plum Farm 1 (cv. Flavour King) (Figure 5.1 C) and two blocks for Plum Farm 2 (cv. Songold) (Figure 5.2 D and E) (Table 5.1), at four points per tree. Four points per tree were sampled from the east, west, inner side and hanging in the drip line zone of the tree. Five samples of three fruit were collected from each point after harvest and before and after packing. Fruit samples were then transported back to the laboratory in a cooling box, as described for water samples and processed within 48 hours after collection.

2.4. Contact surface sample strategy and sampling

Transport swabs with Amies medium (Lasec, Johannesburg) were wetted in the transport medium and then used to sample a 25cm^2 area of contact surfaces in the orchard including hands of pickers, pickers' buckets and transportation crates (Table 5.1, Table 5.4 and Table 5.7). When possible, ten samples were collected from each sampling point. Pickers' equipment is cleaned with water and disinfectant before picking, after lunch and before a new orchard is harvested. In the packhouse the hands of sorters and packers were sampled when possible ten samples were collected for each, as well as fifteen wet wall samples in the pre-cool area, 30 packhouse floor samples and 15 cold room floor and wall samples (Table 5.1, Table 5.4 and Table 5.7). Floor samples were selected throughout the packhouse and the cold room to ensure that high traffic areas were included, for example the entrances and places where fruit could possibly make contact with the floor. The packhouse walls, pre-cool walls and cold room walls and floors are cleaned once a year with water and soap. The bathroom and wash station taps and the processing line (grade-, sort- and pack-line) surfaces were also sampled (Table 5.1, Table 5.4 and Table 5.7). Packhouse floors were cleaned weekly using water and Spore Kill (Hygrotech, South Africa). All packhouse equipment was recorded as being cleaned daily with water and disinfectant. Swab samples were collected and then transported back to the laboratory as described for water samples and processed within one week after collection.

2.5. Bacterial Strains

American Type Culture Collection (ATCC, Manassas, United States of America) cultures, maintained as outlined in section 2.1 of Chapter 3 were used as positive controls for all experiments for the DNA extraction as well as for the Polymerase Chain Reaction.

2.6. Sample processing

All water, fruit and contact surface samples were processed according to Chapter 4's methodology described in section 2.3.

2.7. Statistical Analysis

Statistical analysis was performed as described in Chapter 4 section 2.4.

3. Results

3.1. Plum Farm 1

The rainfall was 24.4 mm ten days prior to the first visit at the time of harvest in January 2007 (South African Weather Services, 2012). Ten days prior to the 2007/2008 season sampling, the rainfall (32.8mm and 34.2mm) was more than in the ten days period prior to the 2006/2007 season as well as ten days prior to the 2008/2009 season (8mm and 0mm) (South African Weather Services, 2012). Between the spray (12 December 2007) and harvest (04 January 2008) sampling period in the 2007/2008 season, 109.4mm of precipitation fell. In contrast, only 39.2mm of precipitation fell between the spray- and harvest period of 2008/2009 (South African Weather Services, 2012). No correlation could be found between the amount of rainfall/precipitation and the degree of contamination (data not included).

During the 2007/2008 and 2008/2009 season there was no significant difference in coliform titres from spray to harvest at all locations sampled (Table 5.2). During the 2008/2009 season, there was no significant difference in coliform counts in water collected from the spray water at the time of spray or at harvest (P= 0.1178) (Table 5.2). Coliform enumeration in 2008/2009 at Dam 1 (P= 0.0002) and Dam 2 (P=0.0081) revealed that coliform titres were significantly higher at the time of spraying (Table 5.2). *Escherichia coli* numbers in 2007/2008 were significantly higher at the time of spraying, when compared to the time of harvest (Table 5.2). During the 2008/2009 spray season, *E. coli* titres from water collected from Dam 1 (P= 0.0693) were not significantly different to those of the later harvest period of the same year (Table 5.2). The *E. coli* numbers during the 2008/2009 spray season were significantly higher than at harvest from water collected from Dam 2 (P=0.0036). However, the *E. coli* counts in water collected from the spray point (P= 0.0001) during the 2008/2009 spray season were significantly lower than the numbers during the harvest season of the same year (Table 5.2).

The coliform counts at the various locations were not significantly different from one another during the 2007/2008 (P=0.3739) and 2008/2009 spray season (Table 5.2). But, the *E. coli* titres in water collected during the 2008/2009 spray season (P= 0.0004) were significantly higher at Dam 2 when compared to Dam 1. The water collected from the spray point, were not significantly different to one another (Table 5.2). Coliform counts in water collected from Dam 1, Dam 2 and the spray point during the 2007/2008 harvest season (P=<0.0001) were not significantly different, but the coliforms titres in the water collected from the wet wall was significantly lower, followed by a significantly lower number in the wash station water (Table

5.2). In contrast, the water samples collected during the 2008/2009 harvest season (*P*=<0.0001) from the spray point and wet walls were significantly higher than that of Dam 1 and Dam 2 (Table 5.2). Dam 1 however had significantly more coliforms than Dam 2 during the 2008/2009 harvest period, with wash station water having no coliforms detected (Table 5.2). *Escherichia coli* titres in water collected from Dam 1 and the spray point were not significantly different, but titres in Dam 2 were significantly lower and no *E. coli* was detected in the wet walls or in the wash station water during the 2007/2008 harvest period (*P*= <0.0001) (Table 5.2). In contrast, wet walls had the highest titre of *E. coli* during the 2008/2009 harvest season, followed by the spray water (Table 5.2). During the 2008/2009 harvest season (*P*= <0.0001), Dam 1 had significantly less *E. coli* present than in the spray water and Dam 2 had significantly less than that (Table 5.2). Again the wash station water during the 2008/2009 harvest season had no *E. coli* O157:H7 that could be detected (Table 5.2).

Coliform titres from water collected from Dam 1 (*P*= 0.3739) and Dam 2 during the spray seasons of 2007/2008 and 2008/2009 were not significantly different (Table 5.2). Coliform titres from water collected from the wash station (*P*=0.2080) and wet wall during the harvest season were not significantly different between 2007/2008 and 2008/2009. However the coliform titres at Dam 1 (*P*= 0.0004) and Dam 2 (*P*=0.0008) were higher in 2007/2008 harvest season than in the 2008/2009 harvest season and the coliforms present in the spray water (*P*=0.003) were higher in 2008/2009 than in 2007/2008 (Table 5.2). The titres of *E. coli* present in Dam 1 (*P*=0.7872), Dam 2 (*P*=0.5467) and the wash station (*P*=0.7872) were not significantly different over the two years (Table 5.2). The *E. coli* titre was higher in 2008/2009 harvest season than in 2007/2008 harvest season in water collected from the spray point (*P*= 0.0002) and the wet walls (Table 5.2).

No *E. coli* O157:H7 or *Salmonella* Typhimurium were detected from the total of 50 water samples collected during both seasons (Table 5.3). Only one water sample, collected from Dam 1, was found to be contaminated with *L. monocytogenes*, resulting in a 6.67% water contamination rate for 2006/2007 and a 2% water contamination rate overall for Plum Farm 1 (Table 5.3). Again, only one water sample, collected from the wet wall water, tested positive for *S. aureus* in 2007/2008 harvest season, resulting in a 4% water contamination rate for the 2007/2008 harvest season and a 2% water contamination rate overall for Plum Farm 1 (Table 5.3).

Table 5.2: Plum Farm 1's Colilert-18® coliform and *Escherichia coli* results of water samples expressed in most probable number per 100ml

Sampling Period	Sampling Point	Season									
		2007/2008		2008/2009			2007/2008		2008/2009		
		Coliforms					*Escherichia coli*				
		MPN[x]		MPN		*P* value	MPN		MPN		*P* value
Water samples at last spray	Dam 1	2332.94	**A** a *A*	2419.60	**A** a *A*	0.3739	76.38	**A** b *A*	13.90	**A** b *B*	0.0060
	Dam 2	2419.60	**A** a *A*	2419.60	**A** a *A*	-	117.02	**A** a *A*	30.04	**A** a *B*	0.0050
	Spray point	ND[y]		2419.60	**A** a	-	ND		10.88	**B** b	-
	***P* value** [z]	0.3139		-			0.0317		0.004		
Water samples at harvest	Dam 1	2332.94	**A** a *A*	842.70	**B** c *B*	0.0004	18.12	**B** a *A*	18.54	**A** c *A*	0.7875
	Dam 2	2419.60	**A** a *A*	1406.80	**B** b *B*	0.0008	11.96	**B** b *B*	10.48	**B** d *A*	0.5467
	Spray point	2419.60	a *B*	2246.30	**A** a *A*	0.0030	16.24	a *A*	85.84	**A** b *A*	0.0002
	Wet Walls	1505.36	b *A*	2419.60	a *A*	0.2080	0.20	c *C*	2419.60	a *A*	-
	Wash station	0.60	c *A*	0.00	d *A*	-	0.00	c *C*	0.00	e *A*	<0.0001
	***P* value**	<0.0001		<0.0001			<0.0001		<0.0001		

[x]: Most probable number per 100 ml; [y]: ND indicates the location was not sampled during the specified season; [z]: *P* value is significant if the value is less than 0.05. Most probable values followed by the same **CAPITAL LETTER** indicate no significant difference between the spray and harvest period within a specific year. Values followed by the same lowercase letter indicate no significant difference at the different locations within a sampling period within one year. Values followed by the same *ITALICISED CAPITAL LETTER* indicate no significant difference at a location between 2007/2008 and 2008/2009.

No *Salmonella* Typhimurium was detected on Plum Farm 1. *Escherichia coli* O157:H7, *L. monocytogenes* and *S. aureus* were detected on Plum Farm 1 (Table 5.5). During 2006/2007 season, *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* were detected (Table 5.5). Samples collected during 2008/2009 did test positive for *E. coli* O157:H7 and *L. monocytogenes* (Table 5.5). *Escherichia coli* O157:H7 was detected on 7.80% of the 153 contact surface samples during 2006/2007. These contact surfaces included; wet wall, pack-, grade-line, packhouse- and cold room- floors (Table 5.5). During the 2007/2008 season *E. coli* O157:H7 was detected on 10.14% of the 148 contact surface samples, with none detected during the 2008/2009 season (37 samples). During the 2007/2008 season *E. coli* O157:H7 was detected on pickers' bags and transportation crates, sorting rollers, disinfectant holder handle, wash station taps, packhouse- and cold room- floors (Table 5.5). During the 2006/2007 and 2007/2008 season most contamination was detected from one sample of the number taken, except for contamination detected on pickers transportation crates during the 2007/2008 season (with two positives detected), packhouse- (eight positive during 2006/2007 season and three during the 2007/2008 season) and cold room- (one positive during the 2006/2007 season and six positives during the 2007/2008 season) floors (Table 5.5). The overall *E. coli* O157:H7 surface contamination rate was 7.99% for Plum farm 1. *Listeria monocytogenes* was only detected from one of the gradeline samples (2006/2007 season), one bathroom tap sample (2006/2007 season), two cold room floor samples (2006/2007 season) and one cold room floor samples (2007/2008 season), resulting in a 2.6% contact surface contamination rate during 2006/2007, a 0.68% contact surface contamination rate during 2007/2008 and a 0% contamination rate for 2008/2009 (Table 5.5). Therefore, an overall contamination rate of 1.48% for all contact surfaces for *L. monocytogenes* on Plum Farm 1 was determined. No *S. aureus* contamination was detected during 2007/2008 and 2008/2009 season. A 5.22% surface contamination rate for *S. aureus* was determined for *S. aureus* during 2006/2007 season with a 2.37% *S. aureus* contact surface contamination rate overall for Plum Farm 1. *Staphylococcus aureus* was detected from three packers' hands, two of the packline samples, one packhouse floor sample and two cold room wall samples (Table 5.5).

3.2. Plum Farm 2

The rainfall on the Plum farm 2 was lower than Plum Farm 1 because it is considered a winter rainfall region. Ten days prior to the first field trip, only 0.6mm of precipitation was recorded (South African Weather Services, 2012). During the 2007/2008 season rainfall increased ten days prior to the sampling period, with 7.8mm falling before the spray period sampling and 3.6mm ten days prior to the harvest sampling (South African Weather

Services, 2012). Between the spray and harvest sampling period a total of 3.6mm of precipitation was recorded (South African Weather Services, 2012). During the 2008/2009 season less precipitation was recorded with 0mm and 0.6mm for the spray and harvest sampling period, respectively (South African Weather Services, 2012). Between the two sampling periods only 0.8mm of rain fell (South African Weather Services, 2012). No correlation could be made between the amount of precipitation and the degree of contamination (data not included).

Coliform counts in water collected from after the trout farm during the 2007/2008 spray season were not significantly different to counts recorded from samples taken during the harvest period of that same year (Table 5.5). Water collected from the inlet to the Dam 1 from the river (P= 0.047), Dam 2 (P=0.0024), and the pesticide point (P=0.0145) had significantly higher titres of coliforms during the 2007/2008 harvest period when compared to the spray period of the same year (Table 5.5). Coliform titres from Dam 3 (P= 0.0463) and Dam 1 (P=0.0019) were significantly higher during the 2007/2008 spray period (Table 5.5). During the 2008/2009 harvest period the coliform numbers were higher when compared to the 2008/2009 spray period from water collected from the inlet to the Dam 1 from the river (P=0.0008) and at Dam 3 (P=<0.0001) (Table 5.5). Water collected from Dam 2 (P=<0.0001), the spray point (P=0.0026) and Dam 1 (P=0.0006) exhibited significantly higher numbers of coliforms in the 2008/2009 spray period than the harvest period (Table 5.5). The coliform titres in water collected after the trout farm (P=0.1419) were not significantly different between the spray and harvest period in 2008/2009 (Table 5.5). The number of *E. coli* enumerated from the inlet to the trout farm was not significantly different from 2007/2008 spray period to 2007/2008 harvest period (P= 0.0689), nor from the 2008/2009 spray period and harvest period (P=0.0637) (Table 5.5). *Escherichia coli* titres in water collected from Dam 2 (P=0.0037), Dam 3 (P=0.0048) and the spray point (P=0.0189) were significantly higher in 2007/2008 harvest period when compared to the 2007/2008 spray period, but water collected from after the trout farm (P= 0.0008) and Dam 1 (P<0.0001) were significantly higher during the 2007/2008 spray period (Table 5.5). The *E. coli* titres in water collected from Dam 2 (P=0.0001), Dam 3 (P=0.0005) and the spray point (P=0.0339) were higher during the spray period of 2008/2009 than the harvest period. However, water collected after the trout farm demonstrated a reverse trend. The *E. coli* titres in water from the inlet to the Dam 1 from the river (P=0.0637) and Dam 1 (P=0.0892) were not significantly different during 2008/2009 spray and harvest season (Table 5.5). As for the 2007/2008 season, water collected after the trout farm were significantly higher at the time of spray than at the time of harvest (P=0.0019) (Table 5.5).

Table 5.3: The number of samples tested positive for a specific organism using the multiplex PCR from water samples collected on Plum Farm 1

Sample location	*Escherichia coli* O157:H7			*Listeria monocytogenes*			*Staphylococcus aureus*		
	2006/2007	2007/2008		2006/2007	2007/2008		2006/2007	2007/2008	
	Harvest	Spray	Harvest	Harvest	Spray	Harvest	Harvest	Spray	Harvest
Dam 1 (n^x=15)	0^y	0	0	1	0	0	0	0	0
Dam 2 (n=15)	0	0	0	0	0	0	0	0	0
Spray point (n=5)	ND^z	ND	0	ND	ND	0	ND	ND	0
Wet wall water (n=10)	0	ND	0	0	ND	0	0	ND	1
Wash station water (n=5)	ND	ND	0	ND	ND	0	ND	ND	0

^{x}: Indicates the total number of samples collected for all sampling periods from each sample location with five replicates collected per sampling period; ^{y}: Indicates the number of samples in which organisms were detected; ^{z}: ND indicates the location was not sampled during the specified season.

Table 5.4: The number of samples tested positive for a specific organism on Plum Farm 1 using the multiplex PCR from samples taken at harvest

Sample Location	*Escherichia coli* O157:H7			*Listeria monocytogenes*			*Staphylococcus aureus*		
	2006/2007[a]	2007/2008[b]	2008/2009[c]	2006/2007	2007/2008	2008/2009	2006/2007	2007/2008	2008/2009
Trees (n^x=40)	0[y]	0	ND[z]	3	0	ND	0	0	ND
Fruit before (n=10) or after packing (n=10)	0	0	ND	0	0	ND	0	0	ND
Pickers' hands (n=20)	0	0	ND	0	0	ND	0	0	ND
Pickers' bags (n=20)	0	1	ND	0	0	ND	0	0	ND
Pickers' transportation crates (n=20)	0	2	ND	0	0	ND	0	0	ND
Wet wall (n=18)	1	0	ND	0	0	ND	0	0	ND
Packers' hands (n=25)	0	0	0	0	0	0	3	0	0
Packline (n=30)	1	0	0	0	0	0	2	0	0
Sorters' hands (n=9)	0	0	ND	0	0	ND	0	0	ND
Sorting rollers (n=23)	0	1	0	0	0	0	0	0	0
Gradeline (n=12)	1	0	ND	1	0	ND	0	0	ND
Disinfectant holder handle (n=4)	ND	1	0	ND	0	0	ND	0	0
Wash station taps (n=13)	0	1	0	0	0	0	0	0	0
Bathroom taps (n=8)	0	0	ND	1	0	ND	0	0	ND
Packhouse floors (n=69)	8	3	0	0	0	0	1	0	0
Cold room walls (n=30)	0	0	ND	0	0	ND	2	0	ND
Cold room floors (n=30)	1	6	0	2	1	0	0	0	0
Total samples positive	**11 (5.95%)**	**9 (5.06%)**	**0**	**7 (3.78%)**	**1 (0.56%)**	**0**	**6 (3.24%)**	**0**	**0**

[x]: Indicates the total number of samples collected for all sampling periods from each sample location; [y]: Indicates the number of samples in which organisms were detected; [z]: ND indicates the location was not sampled during the specified season; [a]: During 2006/2007 season 185 samples were collected; [b]: During 2007/2008 season 178 samples were collected; [c]: During 2008/2009 season 37 samples were collected as a spot check.

Coliform counts enumerated from water collected during the 2007/2008 spray season were all significantly different (*P*<0.0001) (Table 5.5). Water collected after the trout farm had the highest titre of coliforms, followed by the Dam 3, then the inlet to dam 1, then Dam 1, then the pesticide spray point and finally Dam 2, which had the lowest titre of coliforms (Table 5.5). During the 2008/2009 spray season the water collected after the trout farm and from Dam 2 were not significantly different, but Dam 3 had significantly lower coliforms than the previous two points (*P*<0.0001*)* (Table 5.5). The inlet to the trout farm and Dam 1 (which were not significantly different) were significantly different to Dam 3 with the pesticide spray point containing the lowest number of coliforms (*P*<0.0001*)* (Table 5.5). During the 2007/2008 harvest season a different trend was observed with the water collected after the trout farm containing the highest number of coliforms, followed by Dam 3 and the inlet to the trout farm, and then Dam 2 and the pesticide point and the lowest being Dam 1 and the wash station (*P*<0.0001) (Table 5.5).

During the 2008/2009 harvest season a different trend was observed with water collected from Dam 3 and after the trout farm not being significantly different, followed by the wash station water which in turn contained significantly higher titres than the pesticide point and the inlet to the trout farm (*P*<0.0001*)* (Table 5.5). Dam 2 and Dam 1 contained the lowest number of coliforms and were not significantly different during the 2008/2009 harvest period (*P*<0.0001*)* (Table 5.5). The *E. coli* titres during the 2007/2008 spray period were the highest in water collected from after the trout farm and Dam 1, followed by a significant decrease in titre in water collected at the inlet to Dam 1 (*P*<0.0001*)* (Table 5.5). Dam 3 and the pesticide spray point were not significantly different but were significantly lower than the inlet to Dam 1 and the pesticide point and Dam 2 were not significantly different, but Dam 2 and Dam 3 were significantly different (*P*<0.0001*)* (Table 5.5). During the 2008/2009 spray season, water collected from Dam 3 had the highest number of *E. coli*, followed by the inlet to Dam 1 and then Dam 2, the spray point, water collected after the trout farm and Dam 1, which were significantly lower than the inlet to Dam 1 (*P*<0.0001*)* (Table 5.5). As during the 2007/2008 spray season, water collected after the trout farm contained the highest number of *E. coli*, followed by the inlet to Dam 1, Dam 3 and the pesticide spray point (*P*<0.0001*)* (Table 5.5). Dam 2 and the wash station did not differ significantly during the 2007/2008 harvest period, neither did Dam 1 and the wash station (*P*<0.0001*)* (Table 5.5). Again during the 2008/2009 harvest season water collected after the trout farm contained *E. coli* titres which were significantly higher than the other water samples collected (*P*<0.0001*)* (Table 5.5). The *E. coli* titres in water collected during the 2008/2009 harvest season from the inlet to the Dam 1 were significantly lower than water collected after the trout farm, but where significantly

higher than in water collected from Dam 3, followed by Dam 1, Dam 2, the spray point and the wash station (which were all four not significantly different) (*P*<0.0001*)* (Table 5.5).

Coliforms detected in water collected after the trout farm and from the spray point (*P=0.402*) did not differ significantly between the two spray seasons (Table 5.5). Coliform titres were significantly higher in 2007/2008 compared to the following season in water collected from the inlet to Dam 1 (*P=0.0002*), Dam 1 (*P=0.0233*) and Dam 3 (*P=0.0011*) (Table 5.5). Coliforms enumerated in water collected from Dam 2 was significantly higher in the 2008/2009 spray season compared to the previous season (*P<0.0001)* (Table 5.5). Similarly coliforms enumerated from the water collected after the trout farm was not significantly different from 2007/2008 harvest season to 2008/2009 harvest season (*P=0.1419*), neither was the coliform titre from water collected from Dam 1 (*P=0.1506)* (Table 5.5). Coliforms enumerated from water collected in 2007/2008 harvest season were significantly higher than in 2008/2009 at the inlet to Dam 1 (*P=0.0013)*, Dam 2 (*P=0.0034*) and the spray point (*P=0.0003)* (Table 5.5). Coliforms enumerated from Dam 3 (*P= 0.0024)* and the wash station (*P=0.0242*) were significantly higher in 2008/2009 harvest season than in the 2007/2008 harvest season (Table 5.5). *Escherichia coli* titres in water collected from the inlet to Dam 1 (*P=0.0001*), after the trout farm (*P=0.*0008) and Dam 1 (*P=0.0001*) were significantly higher in the 2007/2008 spray season than in the 2008/2009 spray season (Table 5.5). Water collected from the pesticide spray point did not demonstrate to be significantly different between years at the time of spray (*P=0.721*). *Escherichia coli* enumerated from Dam 2 (*P=0.*0001) and Dam 3 (*P=0.0009*) were significantly higher in 2008/2009 spray season than in 2007/2008 spray season (Table 5.5). In contrast, during the harvest season of 2007/2008 and 2008/2009, *E. coli* titres from Dam 1 (*P= 0.0705),* Dam 2 (*P=0.1596*), Dam 3 (*P=0.4629*) and the wash station (*P=0.3138*) were not significantly different (Table 5.5). *Escherichia coli* titres were higher in 2008/2009 harvest season than in 2007/2008 harvest season in water collected from the inlet to the Dam 1 (*P=0.*0064) and after the trout farm (*P=0.037*), conversely *E. coli* enumerated from water collected from the pesticide spray point (*P=0.0042*) was higher during the 2007/2008 harvest season than the 2008/2009 harvest season (Table 5.5).

No *L. monocytogenes* or *Salmonella* Typhimurium was detected in the 131 water samples tested in this study (Table 5.6). Three waters samples, collected from the inlet to Dam 1 from the river were contaminated with *E. coli* O157:H7 during the 2007/2008 spray period, resulting in a 8.6% water contamination rate for 2007/2008 spray period and a 2.3% water contamination rate overall for Plum Farm 2 (Table 5.6). Again, only three water samples, collected from Dam 2, tested positive for the presence of *S. aureus* in the 2006/2007 season,

Table 5.5: Plum Farm 2's Colilert-18® coliform and *Escherichia coli* results of water samples expressed in most probable number per 100ml

Sampling Period	Sampling Point	Season					
		2007/2008	2008/2009		2007/2008	2008/2009	
		Coliforms			*Escherichia coli*		
		MPN[x]	MPN	*P* value	MPN	MPN	*P* value
Water samples at last spray	Inlet to Dam 1 from river	1123.78 **B** c *A*	377.94 **B** c *B*	0.0002	22.52 **A** b *A*	37.44 **A** b *B*	0.0198
	Water after Trout farm	2419.60 **A** a *A*	2419.60 **A** a *A*	-	50.96 **A** a *A*	8.42 **B** c *B*	0.0008
	Dam 1	871.02 **A** d *A*	385.78 **A** c *B*	0.233	50.52 **A** a *A*	3.56 **A** c *B*	0.0001
	Dam 2	7.52 **B** f *B*	2419.60 **A** a *A*	<0.0001	0.60 **B** d *B*	9.98 **A** c *A*	0.0001
	Dam 3	1647.80 **A** b *A*	684.64 **B** b *B*	0.0011	8.84 **B** c *B*	100.92 **A** a *A*	0.0009
	Spray point	334.08 **B** e *A*	283.68 **A** d *A*	0.402	4.84 **B** cd *B*	5.70 **A** c *A*	0.721
	***P* value**[y]	<0.0001	<0.0001		<0.0001	<0.0001	
Water samples at harvest	Inlet to Dam 1 from river	1415.80 **A** b *A*	582.40 **A** c *B*	0.0013	14.63 **A** b *B*	27.70 **A** b *A*	0.0064
	Water after Trout farm	2419.60 **A** a *A*	1986.30 **B** a *A*	0.1419	21.04 **A** a *B*	34.36 **A** a *A*	0.0037
	Dam 1	50.40 **B** d *A*	39.80 **B** d *A*	0.1506	0.00 **B** d *A*	0.60 **B** d *A*	0.0705
	Dam 2	599.70 **A** c *A*	113.20 **B** d *B*	0.0034	5.70 **A** c *A*	4.14 **B** d *A*	0.1596
	Dam 3	1379.20 **B** b *B*	2419.60 **A** a *A*	0.0024	13.70 **A** b *A*	15.68 **B** c *A*	0.4629
	Spray point	593.60 **A** c *A*	582.45 **B** c *B*	0.0003	13.18 **A** b *A*	0.80 **B** d *B*	0.0042
	Wash station	45.10 d *B*	1215.10 b *A*	0.0242	1.42 cd *A*	2.86 d *A*	0.3138
	***P* value**	<0.0001	<0.0001		<0.0001	<0.0001	

[x]: Most probable number per 100 ml. [y]: *P* value is significant if the value is less than 0.05. Most probable values followed by the same **CAPITAL LETTER** indicate no significant difference between the spray and harvest period within a specific year. Values followed by the same lowercase letter indicate no significant difference at the different locations within a sampling period within one year. Values followed by the same *ITALICISED CAPITAL LETTER* indicate no significant difference at a location between 2007/2008 and 2008/2009.

Table 5.6: The number of samples tested positive for a specific organism using the multiplex PCR in water samples collected on Plum Farm 2

Sample location	*Escherichia coli* O157:H7			*Listeria monocytogenes*			*Staphylococcus aureus*		
	2006/2007	2007/2008		2006/2007	2007/2008		2006/2007	2007/2008	
	Harvest	Spray	Harvest	Harvest	Spray	Harvest	Harvest	Spray	Harvest
Inlet to Dam 1 from river (n[x]=15)	0[y]	3	0	0	0	0	0	0	0
Water after trout farm (n=15)	0	0	0	0	0	0	0	0	0
Dam 1 (n=15)	0	0	0	0	0	0	0	0	0
Dam 2 (n=15)	0	0	0	0	0	0	3	0	0
Dam 3 (n=15)	0	0	0	0	0	0	0	0	0
Spray point (n=15)	0	0	0	0	0	0	0	0	0
Wash station (n=10)	0	ND[z]	0	0	ND	0	0	ND	0

[x]: Indicates the total number of samples collected for all sampling periods from each sample location with five replicates collected per sampling period; [y]: Indicates the number samples tested positive for a specific organism; [z]: ND indicates the location was not sampled during the specified season.

Table 5.7: The number of samples tested positive for a specific organism on Plum Farm 2 using the multiplex PCR from samples taken at harvest

Sample Location	*Escherichia coli* O157:H7		*Listeria monocytogenes*		*Staphylococcus aureus*	
	2006/2007[a]	2007/2008[b]	2006/2007	2007/2008	2006/2007	2007/2008
Trees (n[x]=80)	0[y]	0	0	0	0	0
Fruit before (n=8) or after packing (n=8)	0	0	0	0	0	0
Pickers' hands (n=10)	0	0	0	0	0	0
Pickers' bags (n=10)	0	1	0	0	0	0
Pickers' transportation crates (n=17)	0	0	2	0	0	0
Bathroom taps (n=20)	0	0	0	0	0	0
Wash station taps (n=8)	1	0	0	0	0	0
Grading bins (n=19)	0	1	0	0	0	0
Brushes (n=22)	0	0	0	0	0	0
Sorting rollers (n=20)	0	0	1	0	0	0
Sortline (n=18)	0	0	0	0	0	0
Sorters' hands (n=20)	0	1	0	0	0	0
Packline (n=19)	0	0	0	0	0	0
Packers' hands (n=20)	1	0	0	0	0	0
Cold room floors (n=30)	0	0	0	0	0	0
Cold room walls (n=30)	0	0	0	0	0	0
Packhouse floors (n=60)	5	0	0	0	0	0
Total samples positive	**7 (3.43%)**	**3 (1.39%)**	**3 (1.47%)**	**0**	**0**	**0**

[x]: Indicates the total number of samples collected for all sampling periods from each sample location; [y]: Indicates the number of samples tested positive for a specific organism;

[a]: During 2006/2007 season 204 samples were collected; [b]: During 2007/2008 season 216 samples were collected.

resulting in a 8.3% water contamination rate for 2006/2007 and a 2.3% water contamination rate overall for Plum Farm 2 (Table 5.6).

No *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* was detected from the 90 fruit samples collected from Plum Farm 2, resulting in a 0% fruit contamination for the farm (Table 5.7).

No *Salmonella* Typhimurium or *S. aureus* were detected from contact surface samples collected on Plum Farm 2 (Table 5.7). *Escherichia coli* O157:H7 was however detected from 4.43% of contact surface samples during 2006/2007 and from 1.81% of samples in 2007/2008 season, resulting in a 3.09% overall *E. coli* O157:H7 contact surface contamination. During 2006/2007 season five samples collected from the packhouse floor were contaminated with *E. coli* O157:H7 and one of the wash station taps was also contaminated (Table 5.7). One packer's hand was contaminated with *E. coli* O157:H7 (Table 5.7). In contrast with the 2007/2008 season results one picker's bag, one grading bin and one sorter's hand was contaminated with *E. coli* O157:H7 (Table 5.7). No *L. monocytogenes* was detected from contact surface samples during 2007/2008 season. However during 2006/2007 season, two pickers' transportation crates and one sorting roller was found to be contaminated with *L. monocytogenes* (Table 5.7). The overall *L. monocytogenes* surface contamination rate for Plum Farm 2 was 0.93%, with a 1.90% surface contact contamination rate during 2006/2007 season (Table 5.7).

4. Discussion

Accurate scientific data is required in order to determine the true levels of risks of specific hazards that are associated with fresh produce within the fresh produce chain. Of the hazards that could potentially be associated with fresh produce, no *Salmonella* Typhimurium was detected on either of the plum farms. But, *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* were detected on both farms from varying samples over the entire study period. The lack of detection of *Salmonella* Typhimurium within the samples collected over the entire sampling period demonstrated that a zero incidence of this serovar was determined within the specific samples, when compared to the other organisms studied. The absence of this specific *Salmonella* serovar within the samples collected does however not indicate the total absence of all the *Salmonella* species in the studied environment. Environments can be contaminated with *Salmonella*, Duffy *et al.* (2005) detected the presence of *Salmonella* species in canal (7.5% of 80 samples tested), furrow (10% of 20 samples tested) and

reservoir (26.67 of 30 samples tested) water samples as well as from various environmental contact surfaces, including boxing ramps (6.67% of 30 samples tested), conveyer belt (1.42% of 70 samples tested), conveyer rollers (10% of 20 samples tested) and the unloading ramp (6.67% of 15 samples tested).

Listeria monocytogenes was detected from fruit samples from only one farm. The total percentage of fruit contaminated with *L. monocytogenes* over the two seasons on the one farm was 5.3%. A total (fruit, contact surface and water samples) contamination rate of 2% for *L. monocytogenes* was determined for Plum Farm 1 which was similar to that demonstrated for Peach Farm 1, which is in the same region of the country (Chapter 4). Peach Farm 2 (Chapter 4) and Plum Farm 2 also exhibited similar *L. monocytogenes* total contamination rates with no *L. monocytogenes* detected in the samples collected from the two farms. *Listeria monocytogenes* has also been previously detected from fresh produce samples by Johannessen *et al.* (2002) and Pingulkar *et al.* (2001). Johannessen *et al*. (2002) reported a 3% (198 samples tested) fresh produce contamination rate for *L. monocytogenes* and Pingulkar *et al.* (2001) demonstrated a 10% (116 samples tested) fruit contamination rate for *L. monocytogenes,* both these values are comparable to the fruit contamination rate of one of the plum farms which was demonstrated in this study, even though total fruit samples were lower. Both peach farms (Chapter 4) did demonstrate contamination of fruit with *E. coli* O157:H7, *L. monocytogenes* and *S. aureus.* A similar trend was not found for the plum farms assessed in this study. Cooley *et al.* (2007), Johnston *et al.* (2005) and Mukherjee *et al.* (2004) did not detect *E. coli* O157:H7, Riordan *et al.* (2001) and Selma *et al*. (2007) did detect *E. coli* O157:H7 on fresh produce surveyed in their study and Johannessen *et al.* (2002) did detect the presence of *S. aureus* on fresh produce.

Escherichia coli O157:H7, *L. monocytogenes* and *S. aureus* should not be present on contact surfaces if proper and regular personal hygiene and facility sanitation are implemented. *Escherichia coli* O157:H7 was detected on 7.99% of contact surface samples tested over three years from Plum Farm 1, *L. monocytogenes* from 1.47% of contact surface samples from the same farm and *S. aureus* from 2.37% of contact surface samples. From Plum Farm 2 no *S. aureus* could be found, only 0.93% of contact surface samples demonstrated the presence of *L. monocytogenes* and 3.09% were positive for *E. coli* O157:H7. *Escherichia coli* was detected previously from 9.3% (n=210) of contact surface samples tested in a study by Duffy *et al.* (2005) and from the Peach Farms investigated (Chapter 4) in this study. *Escherichia coli* O157:H7 contact surface contamination rate was recorded as 6.8% for both plum farms under investigation, which is comparable to the surface contamination rate of both peach farms. *Escherichia coli* O157:H7 was detected

from a picker's bag, a picker's trolley, wet wall, bathroom tap, disinfectant handle, sorting rollers, gradeline, packline, packer's hand, sorter's hand, packhouse floors and cold room floors collectively from the plum farms, similar to that found by Duffy *et al.* (2005). Previous studies have demonstrated that *E. coli* O157:H7 can be transferred to the contact surfaces from hands or other contact surfaces following the interaction of these surfaces (Smith, 2007). *Listeria monocytogenes* was detected from a picker's bag, sorting roller, gradeline, bathroom tap and cold room floors. *Listeria monocytogenes* is a known soilborne organisms and Smith (2007) has demonstrated that *L. inocula* (a *L. monocytogenes* surrogate) is able to be transferred from hands to contact surfaces, it is therefore likely that *L. monocytogenes* could have entered the production line due to contact with soil in the orchard or from hands of handlers, even though hands were not found to be contaminated. *Staphylococcus aureus* was detected from packers' hands, packline, packhouse floors and cold room walls. Smith (2007) demonstrated that *S. aureus*, a natural inhabitant of the human skin (Adams and Moss, 2000) was capable of being transferred from workers hands to contact surfaces. Both plum farms had a high contamination rate of *E. coli* O157:H7 on packhouse floors. The contamination of Plum farm 1's packhouse floor was highest during the 2006/2007 season where 26.67% of packhouse floor samples tested positive for *E. coli* O157:H7 contamination. This floor contamination rate decreased to 10% from the same sampling area in the subsequent season and then to not detectable from the same sampling area in the 2008/2009 season. During the 2006/2007 season it was observed that geese were free to walk around outside the packhouse, in the subsequent season all geese had been moved to an alternative farm. Wallace *et al.* (1997) found that *Escherichia coli* O157:H7 was present in a small number (13; n= 200) of wild birds, mainly gulls. Wallace *et al*. (1997) however expressed concern for the potential of these birds to further infect cattle that come into contact with the contaminated faeces causing a chronic infection of a farm's wildlife and livestock. A large amount of bird faeces was observed deposited around the packhouse during the first visit (2006/2007). Brackett (1999) and Tauxe *et al.* (1997) have demonstrated that *E. coli* O157:H7 is able to survive in the soil for months to years. Bacteria, such as *E. coli* O157:H7 can be transferred from the origin to destinations as far as the participant's households (Cleaning Industry Research Institute Staff, 2011). Therefore, the *E. coli* O157:H7 detected from the packhouse floors on both farms could have been carried into the packhouse on the soles of worker's shoes entering the packhouse.

A 2% *L. monocytogenes* water contamination rate was determined for Plum Farm 1 and a 2% *S. aureus* water contamination rate overall. Water from Dam 1 was contaminated with *L. monocytogenes*, contamination could have occurred from soil. Selma *et al.* (2007) also found that *Listeria* spp. contaminated creek, pond and irrigation water sampled and the

authors surmised that the contamination occurred because the organisms are present in soil and are associated with decomposed organic matter (Dowe *et al.*, 1997; Porto and Eiroa, 2001). In this study it was found that the wet wall water was contaminated with *S. aureus* and it was observed that the collection drum for the wet wall water was open outside. *Staphylococcus aureus*, a human skin inhabitant, contamination occurred on Plum Farm 2 in three of Dam 2's samples, this dam is also used for recreational activities. Water collected from the river at the beginning of the water collection scheme on Plum Farm 2 was contaminated with *E. coli* O157:H7. Even though water entering the farm's water collecting scheme was contaminated with *E. coli* O157:H7, no *E. coli* O157:H7 contamination was determined further downstream. Izumi *et al.* (2005) demonstrated the presence of *E. coli* O157:H7 in the agriculture water and Keene *et al.* (1994) also detected the presence of *E. coli* O157:H7 from Oregan lake.

According to the South African DWAF (1996) and to the WHO (2006) faecal coliform levels must not exceed 1000 counts per 100ml for the safe use of water or wastewater for irrigation. Faecal coliforms are traditionally used as indicators of faecal contamination. All water in this study can be used as irrigation water provided fruit are irrigated using drip irrigation, preventing the fruit from getting wet during the process of irrigation. Water used in the packhouse, for example in a wet wall system or water used for hygiene practices, should be potable water (Kirby *et al.*, 2003). Water used in Plum Farm 1's wash station was found to be of good quality, however water used for circulation through the wet walls of Plum Farm 1 and the wash station of Plum Farm 2 was not found to be potable at the time of this study and if the contamination persists the water should be further purified (SANS 241:2005). Water used in the packhouse should be controlled better and it is easier to control the quality of this water when compared to the surface water (Kirby *et al.*, 2003; Odumeru *et al*., 1999). Surface water is easily contaminated by human activity as is evident with the presence of *S. aureus* in the wet wall system on Plum Farm 1 and the contamination with *S. aureus* of Plum Farm 2's Dam 2. Water tested over the two seasons during the spray and harvest period of each season were not comparable to the contamination that Gemmell and Schmidt (2011) found in a similar river in South Africa, which the authors compared to titres found in raw sewage.

In conclusion, no *Salmonella* Typhimurium was detected in any samples collected from both plum farms. Reacting on the initial research report, both farms improved their facility and personal hygiene programmes during 2007 reflecting a decrease in contamination rates for contact surfaces. Only *L. monocytogenes* was found to still be prevalent on fruit samples collected from Plum Farm 1. Water contamination rate of *E. coli* O157:H7, *L.*

monocytogenes and *S. aureus* were all 2% for both plum farms. Water tested complies with irrigation water standards (WHO, 2006), as the *E. coli* titres ranged from the highest 117.96 to lowest 0, all within the requirements. Water quality of the water used in the packhouse for cooling and personal hygiene for both farms should in future be improved.

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Chapter 6

Growth dynamics of *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* under different nutrient and temperature conditions

Stacey Collignon and Lise Korsten

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Abstract

Foodborne pathogens potentially associated with fresh produce can cause a number of diseases that affect the well-being of consumers. An important control factor in food safety assurance systems is the use of temperature to regulate and control the growth of these microorganisms. In the stone fruit supply chain, the fruit and contact surfaces are exposed to three temperatures, 0.5^{o}C, 4^{o}C and 21^{o}C. If these foodborne pathogens are able to survive and grow at these temperatures then control strategies need to be improved to prevent contamination of fresh produce and contact surfaces. The aim of this study was to determine if temperature plays a role in the growth dynamics of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* type cultures inoculated on fruit surfaces, under different nutrient conditions and on nutrient free surfaces using tiles as an environment surface example. Prepared cultures of *E. coli* O157:H7*, L. monocytogenes, Salmonella* Typhimurium and *S. aureus* were used to inoculate peaches, plums, nutrient rich TSB, nutrient poor TSB and tiles. Once inoculated, the fruit and broth were incubated at fluctuating temperatures and the broths and tiles were incubated at either 0.5^{o}C, 4^{o}C or 21^{o}C all for six days to determine the growth dynamics of the four pathogens under different conditions. All pathogens grew in broths under fluctuating incubation temperature, but on peaches the pathogen titres remained constant and on plums the titres decreased. All pathogens either remained constant or decreased in titre when incubated at 0.5^{o}C in the nutrient- rich and -poor broths, except *L. monocytogenes* which was able to grow at 0.5^{o}C. All pathogens were able to grow at 21^{o}C and all pathogen titres remained constant or increased at 4^{o}C when the nutrient content of the broth was optimal. All four pathogens were unable to grow but were able to

survive on the tile surfaces. It is imperative that contamination of fruit and contact surfaces be prevented because survival and growth is possible at specific temperatures.

1. Introduction

Haemorrhagic *Escherichia coli* is an organism, often foodborne due to faecal contamination that can lead to a haemorrhagic colitis, haemolytic uremic syndrome (Mead and Griffin, 1998) or thrombotic thrombocytopenia purpurea (Willshaw *et al.*, 2000). All three of these diseases often result in hospitalisation and the organism is therefore considered a moderate foodborne pathogen (Mataragas *et al.*, 2008; Sumner *et al.*, 2005). *Escherichia coli* is an organism that can survive at any temperature ranging from 7^{o}C to 50^{o}C, this organism can even survive at refrigeration and freezing temperatures for a period of time (Adams and Moss, 2000; Willshaw *et al.*, 2000). Unlike *E. coli* O157:H7, *Listeria monocytogenes* can grow at refrigeration temperatures (Adams and Moss, 2000; Farber and Peterkin, 2000). *Listeria monocytogenes* is a psychrotrophic organism that can grow at temperatures as low as 0^{o}C, even if nutrients are scarce. *Listeria monocytogenes* can cause a mild disease to a severe meningitis and meningoencephalitis especially in patients with a compromised immune system (Bremer *et al.*, 2003).

Salmonella species cause a wide range of gastrointestinal infections ranging from an asymptomatic carrier to severe diarrhoea (Adams and Moss, 2000). *Salmonella* species are not psychrotrophic organisms, but rather grow at temperatures between 5^{o}C and 48^{o}C, some *Salmonella* spp. are able to survive freezing temperatures, depending on the physiology of the organisms (D'Auost, 2000). Similarly, *S. aureus* only grows at temperatures between 7^{o}C and 48^{o}C and is able to survive freezing temperatures. *Staphylococcus aureus* is classified as a minor foodborne pathogen (Mataragas *et al.*, 2008; Sumner *et al.*, 2005) and is an indicator of poor personal hygiene, however *S. aureus* can cause a range of infections, among these are cutaneous infections, organ infections and toxicoses (Novick *et al.*, 2001). *Staphylococcus aureus* can also survive on surfaces with little or no nutrients as this organism is adapted to surviving on the human skin (Adams and Moss, 2000). Temperature plays an important role in the growth, survival and death of an organism. Storage temperatures can aid in the elimination of foodborne pathogens and therefore add to the assurance of food safety.

Storage temperatures differ depending on the commodity. Most consumers store food in a refrigerator (4^{o}C to 7^{o}C). Fruit are stored in the consumer's home either at room temperature

or in the fridge. Other temperatures that play a role in the fruit supply chain are the on-farm storage, packhouse or processing environment, transportation and at retail. During the stone fruit supply chain, fruit are picked and stored at 0.5^{o}C for no longer than four days on the farm in cold storage facilities. From the farm, fruit are transported in refrigerated road transport vehicles (4^{o}C) to the distributor or to the retailer where the product will be retained at 4^{o}C until display and sales. The distributor either repacks produce or directly distributes the product to local stores. Fruit are then bought by consumers and stored either at room temperature (21^{o}C) or at refrigeration temperatures. All areas where fruit are stored have contact surfaces that might become contaminated and allow the survival of organisms that can potentially lead to product contamination.

Surfaces within the food production arena can harbour microorganisms (Duffy *et al*., 2005; Chapter 4 and Chapter 5). Studies have demonstrated that foodborne bacterial pathogens do not need a constant high nutrient source to survive for extended periods (Kusumaningrum *et al.*, 2003). Presence of these foodborne pathogens within the food production arena might lead to the contamination of foods and of contact surfaces in such environments. *Escherichia coli, L. monocytogenes* and *S. aureus* have been shown to be transferred to contact surfaces and food through exposure to other contaminated surfaces or hands. Floors can also become contaminated with microorganisms being carried in on the soles of shoes of people moving through the food production or storage environments (Cleaning Industry Research Institute Staff, 2011). The presence and survival of these organisms, as has been demonstrated for *L. monocytogenes*, within a packhouse and cold storage facility can lead to biofilm formation on the inert surfaces. The formation of biofilms makes it increasingly difficult to remove these microorganisms and can then lead to chronic contamination of the food production environment (Hood and Zottola, 1997). If these biofilms do become dislodged there is a possibility that they are able to end up in or on food products.

The aim of this study was to determine how temperature and fluctuating nutrient conditions affect the growth of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus,* and to determine if these pathogens are able to survive and potentially grow on peaches and plums during simulated local transit conditions.

2. Materials and Methods

2.1. Cultures

American Type Culture Collection cultures namely *E. coli* O157:H7 (ATCC 35150), *L. monocytogenes* (ATCC 19115), *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028) and *Staphylococcus aureus* (ATCC 12600) were used as reference cultures in this study. All cultures were maintained lyophilized and stored at -70^{o}C with subcultures on standard 1 medium (Merck, Johannesburg) prepared 24h prior to use. Cultures were used to inoculate five replicates of 100 ml TSB (Merck) for each pathogen and were subsequently incubated at 37^{o}C for 18h to achieve a concentration of 8 log cfu/ml. Cultures were centrifuged at 5000 r.p.m. and washed twice with sterile distilled water and finally re-suspended into 1% (w/v) Peptone Buffered Water (Merck). Cultures were also serially diluted to obtain an inoculum concentration of 7 log for fruit inoculation to achieve a concentration of 5 log cfu/cm^{2}. Cultures were further diluted to obtain a concentration of 6 log cfu/ml, which was used to inoculate the nutrient -rich and -poor broths to achieve a 3 log cfu/ml concentration which would achieve a final concentration of 3 log cfu/ml. In addition, cultures that were serially diluted to 5 log cfu/ml were used to inoculate a 12.25cm^{2} section of a floor tile to achieve a final concentration of 3 log cfu/section to determine the survival of each pathogen on a nutrient free surface. Concentrations were confirmed by serial dilution and subsequent plating in duplicate.

2.2. Growth of foodborne pathogens under various growth conditions

2.2.1. Nutrient rich and nutrient poor

Forty-eight sterile 100 ml TSBs, (consisting of 1.5g of peptone and 0.5g of sodium chloride per 100 ml) called nutrient rich broths and 48 sterile 0.3 (w/v) TSB (consisting of 0.15g of peptone and 0.05g of sodium chloride per 100ml) called nutrient poor were both divided into four sets of twelve. Three of the twelve were inoculated each with 500 µl of 6 log concentration of one pathogen, resulting in three replicates with *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus*. Following inoculation the final concentration of each culture in the broth was confirmed to be 3 log cfu/ml. Each set was subsequently incubated at 0.5^{o}C, 4^{o}C, 21^{o}C and at fluctuating temperatures for six days, with daily agitation. Fluctuating temperatures were as follows; 21^{o}C for 24 hours (Day 0 to Day 1), 0.5^{o}C for 48 hours (Day 1 to Day 3), again at 21^{o}C for 24 hours (Day 3 to Day 4) and finally at 4^{o}C for 48 hours (Day 4 to Day 6), these fluctuating temperatures represent the local supply chain temperatures for peaches and plums. Daily, 1 ml of the solution was

removed from all 48 broths and serially diluted with subsequent plating onto Baird-Parker medium for *S. aureus*, Oxford Listeria Selective Agar for *L. monocytogenes*, Levine Eosine-Methyl Blue Agar for *E. coli* O157:H7 and XLD Agar for *Salmonella* Typhimurium (all supplied by Merck). Following 24 hour incubation, counts were recorded and transformed to log (x+1) cfu/ml.

2.2.2. Fruit

Peaches (*Prunus persica* cv. Excellence) and plums (*Prunus domestica* cv. Flavour King) were aseptically hand harvested at optimum maturity from two commercial farms in the North-West Province and Limpopo Province, respectively. The full experiment was repeated on two separate occasions. Fruit of a uniform size and weight and without pest, disease or damage were used in this study. Harvested fruit were bagged in paper bags and transported to the laboratory in cooler boxes and stored at 4^{o}C overnight (approx. 12 to 15 h). Collected fruit consisted of 24 peaches (five replicates for four time intervals selected and four negative controls) and 24 plums (five replicates for four time intervals and four negative controls) were used to quantify the pathogen titre following inoculation. Fruit were all washed using 0.05% (v/v) Sodium hypochlorite for 30s, rinsed twice with sterile distilled water and allowed to air drying before inoculation.

Spot inoculation, using 50µl of culture, for the quantification of pathogen titres was carried out on five fruit per time interval (0d, 1d, 4d, 6d). Spot inoculation was carried out ensuring that cultures were not mixed. Following spot inoculation the final concentration of each culture on the fruit was confirmed to be 5 log cfu/fruit with serial dilutions as described before. The viable *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* titres remaining on these fruit following various time intervals (0d, 1d, 4d, 6d) was determined. Fruit were incubated overnight at 21^{o}C where after fruit were transferred to -0.5^{o}C for two days to mimic the on-farm storage of fruit. Fruit were then transferred to 21^{o}C to for two days to mimic the transportation and market sale of fruit at room temperatures, fruit were subsequently transferred to refrigeration temperature (4^{o}C) for one day to simulate the home storage conditions of fruit.

Fruit were removed from the incubation storage areas at the different time intervals (0d, 1d, 4d and 6d) and washed to determine the bacterial titre present on the fruit. Fruit were washed in 500ml quarter strength Ringer's solution amended with 0.02% Tween-80 (Sigma, Johannesburg) in an Ultrasonic Bath (Labotec, Johannesburg) for 30s. The Ringer's solution

was subsequently filtered through a 0.45nm nitrocellulose membrane. The membrane was subsequently used for serial dilution and plating in duplicate onto selective agar specific for the four pathogens [Baird-Parker medium for *S. aureus*, Oxford Listeria Selective Agar for *L. monocytogenes*, Levine Eosine-Methyl Blue Agar for *E. coli* O157:H7 and XLD Agar for *Salmonella* Typhimurium] was performed and counts recorded. Volume displaced (vd) for each fruit was recorded and converted to cm^2 using the following equation: $A=4.84[(vd)^{1/3}]^2$ (De Jager, 1999*).* Counts were converted to cfu/cm^2 and transformed to log (x+1) cfu/cm^2.

2.2.3. Nutrient free floor tiles

Two vinyl composition tiles were cut into 3.5 x 3.5 cm blocks. All blocks were dip sterilised in 70% ethanol for 1 min and subsequently allowed to air dry in the laminar flow cabinet. Sixty-three of these tile section blocks were subsequently spot inoculated with 50µl of 5 log cfu/ml of each culture. Spot inoculation was carried out to ensure that cultures did not mix. Following spot inoculation with *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* resulted in a final concentration of 3 log cfu/tile. The concentration of the inoculum was confirmed to be 3 log cfu/tile by serial dilution and plating. The 63 tile blocks were then divided into three sets according to temperature for incubation, placed into sterile storage containers and incubated at 0.5^oC, 4^oC and 21^oC mimicking the temperatures at which peaches and plums are stored, for six days. Three tiles per day per temperature were removed from each temperature and placed into sterile 100 ml containers. Five grams of sterile 0.55µm glass beads (Glass World, Johannesburg) with 1 ml 0.1% peptone buffered water (Merck) was added to the 12.25 cm^2 tile block and subsequently shaken at 5 kHz for 10 minutes. Subsequently 8 ml of 0.1% peptone buffered water was added to the block. The solution was then further diluted as necessary and plated onto selective media as described in section 2.2.1. Following 24 hour incubation, counts were recorded and transformed to log (x+1) cfu/tile.

2.3. Statistical Analysis

All experiments were repeated. Results obtained for each repeat were analysed together, therefore 10 replicates were analysed (two repeats of five replicates). Statistical analysis was performed on log values. Data were analysed using SAS 9.2 for Windows (SAS Institute Inc., Cary United States of America). A one-way analysis of variance was used to determine the difference in pathogen titres present on fruit surfaces. Means were analysed using the least significant difference (using the Fischer test) at a 5% level of significance.

3. Results

3.1. Growth dynamics of foodborne pathogens under fluctuating temperatures

When growing in nutrient rich broth under fluctuating temperature conditions, *E. coli* O157:H7 titres exhibited an overall increase in titres from Day 0 to Day 6 (Figure 6.1; Appendix 1). On Day 1, 2 and 3 the *E. coli* O157:H7 titres where the highest of all six days tested when incubated in a nutrient rich broth under fluctuating temperatures (Figure 6.1; Appendix 1). On the day of inoculation the *E. coli* O157:H7 titres were the lowest of all six days (Figure 6.1; Appendix 1). Following the initial inoculation, broths were moved to 21^{o}C therefore there was the significant increase in titre from Day 0 to Day 1 (Figure 6.1; Appendix 1). Following the constant titre levels on Day 1, Day 2 and Day 3, there was a significant decrease in titre on Day 4 (Figure 6.1; Appendix 1). *Escherichia coli* O157:H7 titres recovered on Day 5 to titre levels comparable to Day 1 and remained constant to Day 6 (Figure 6.1; Appendix 1).

When growing in nutrient poor broth under fluctuating temperature conditions, *E. coli* O157:H7 titres exhibited an overall increase in titres from Day 0 to Day 6 (Figure 6.1; Appendix 1). In nutrient poor broth, *E. coli* O157:H7 titres were at the lowest level on the day of inoculation (Day 0) with a significant increase in titre on Day 1 (Figure 6.1; Appendix 1). *Escherichia coli* O157:H7 titres on Day 1, Day 2, Day 4, Day 5 and Day 6 were not significantly different (Figure 6.1; Appendix 1). From Day 2 to Day 3 there was a significant increase in *E. coli* O157:H7 titre, with a significant decrease in titre from Day 3 to Day 4 (Figure 6.1; Appendix 1).

When growing in a nutrient rich broth under fluctuating temperatures conditions, *L. monocytogenes* titres demonstrated an overall increase from Day 0 to Day 6 (Figure 6.2; Appendix 1). The biggest significant increase in titre of *L. monocytogenes* was recorded after one day in the nutrient rich broth, following the 21^{o}C incubation (Figure 6.2; Appendix 1). The titres recorded on Day 1 were maintained until Day 2, where after there was a significant decrease in *L. monocytogenes* titre recorded on Day 3 due to the 0.5^{o}C incubation (Figure 6.2; Appendix 1). *Listeria monocytogenes* titres recorded on Day 3 were maintained until the completion of the study on Day 6, following a two day incubation at 21^{o}C and a further day at 4^{o}C (Figure 6.2; Appendix 1).

In the nutrient poor broth the titres of *L. monocytogenes* fluctuated according to the temperature and availability of nutrients but there was an overall increase in titres from Day 0 to Day 6. On Day 0, the titre of *L. monocytogenes* in the nutrient poor broth was the lowest

(Figure 6.2; Appendix 1). Following the 21°C incubation from Day 0 to Day 1, titres increased significantly (Figure 6.2; Appendix 1). Interestingly, following the 0.5°C incubation on Day 2 and Day 3, there was a significant increase in *L. monocytogenes* titres to the highest level recorded in nutrient poor broth under fluctuating temperatures for six days (Figure 6.2; Appendix 1). Following Day 3 there was a significant decrease in *L. monocytogenes* titres recorded on Day 4, with a further decrease in titre on Day 5 (Figure 6.2; Appendix 1). *Listeria monocytogenes* titres on Day 5 and Day 6 were not significantly different (Figure 6.2; Appendix 1).

Salmonella Typhimurium demonstrated an overall growth increase from Day 0 to Day 6 when incubated in a nutrient rich broth at fluctuating temperatures (Figure 6.3; Appendix 1). The lowest *Salmonella* Typhimurium titres were recorded on Day 0 (Figure 6.3; Appendix 1). Following the first 24 hour incubations at 21°C there was a significant increase in *Salmonella* Typhimurium titres incubated in the nutrient rich broth (Figure 6.3; Appendix 1). A subsequent significant increase was seen from Day 1 to Day 2 following one day at 0.5°C, titres recorded on Day 2 were the highest recorded over the six-day period (Figure 6.3; Appendix 1). Following an additional 24 hours at 0.5°C titres on Day 3 were significantly lower than on Day 2 (Figure 6.3; Appendix 1). *Salmonella* Typhimurium titres on Day 4 were not significantly different to those on Day 3 (Figure 6.3; Appendix 1). At the following two days at 21°C, titres significantly increased on Day 5 and these titres were maintained to Day 6, after the 24 hours at 4°C (Figure 6.3; Appendix 1).

An overall increase in *Salmonella* Typhimurium titres was recorded from Day 0 to Day 6 incubated at fluctuating temperatures in a nutrient poor broth. *Salmonella* Typhimurium titres increased significantly within the first 24 hours following the 21°C incubation (Figure 6.3; Appendix 1). These titres remained constant until Day 4 following 48 hours of 0.5°C incubation and 24 hours of 21°C incubation (Figure 6.3; Appendix 1). Titres on Day 4 and Day 5 were not significantly different but titres on Day 5 were significantly higher than titres recorded on Day 3 (Figure 6.3; Appendix 1). Following the 24 hour incubation at 4°C the *Salmonella* Typhimurium titres again significantly decreased (Figure 6.3; Appendix 1). Titres on Day 1, Day 2, Day 3, Day 4 and Day 6 were not significantly different (Figure 6.3; Appendix 1).

Titres of *S. aureus* demonstrated an overall increase from Day 0 to Day 6 when incubated at fluctuating temperatures in a nutrient rich broth (Figure 6.4; Appendix 1). The lowest titres for all six days were recorded on Day 0 (Figure 6.4; Appendix 1). Following a 24 hour

incubation at 21^{o}C, *S. aureus* titres reached the highest titre recorded on all six days (Figure 6.4; Appendix 1). Titres recorded on Day 1 and Day 2, following Day 2 titres remained constant until Day 6 (Figure 6.4; Appendix 1).

Staphylococcus aureus titres in a nutrient poor broth incubated at fluctuating temperatures demonstrated a systematic and overall increase from Day 0 to Day 6 (Figure 6.4; Appendix 1). Titres on Day 1 were significantly higher than on Day 0 (Figure 6.4). Day 1 titres were maintained until Day 3 (Figure 6.4; Appendix 1). Titres on Day 4 were significantly higher than titres on Day 1, Day 2 and Day 3 (Figure 6.4; Appendix 1). Titres on Day 5 and Day 6 were not significantly different, but were significantly higher that titres on Day 4 (Figure 6.4; Appendix 1).

3.2. Growth dynamics of foodborne pathogens under constant temperatures

When *E. coli* O157:H7 was incubated at 0.5^{o}C, the highest titres of the six day trial was determined on Day 0, in both the nutrient -rich and -poor broths (Figure 6.1; Appendix 1). When incubated at 0.5^{o}C in nutrient rich broth Day 1 and Day 0 titres did not differ significantly (Figure 6.1; Appendix 1). Day 0 did not differ significantly from days 3 or 4 (Figure 6.1; Appendix 1). Days 2, 5 and 6 did not differ significantly and these titres were significantly lower than the titres on Day 0 (Figure 6.1; Appendix 1). Therefore there was an overall decrease in *E. coli* O157:H7 titres from Day 0 to Day 6 incubated in both a nutrient –rich and –poor broth at 0.5 ^{o}C (Figure 6.1; Appendix 1). In the nutrient poor broth, the titres of *E. coli* O157:H7 followed a decreasing trend with the highest titre determined on Day 0. Following Day 0 there was a significant decrease in titre to Day 1 (Figure 6.1; Appendix 1). Titres recorded on Day 2 were significantly higher than titres on Day 1 but significantly lower than titres on Day 0 (Figure 6.1; Appendix 1). Following Day 2 titres significantly decreased to titres lower titres on Day 3 and remained unchanged until Day 6 (Figure 6.1; Appendix 1).

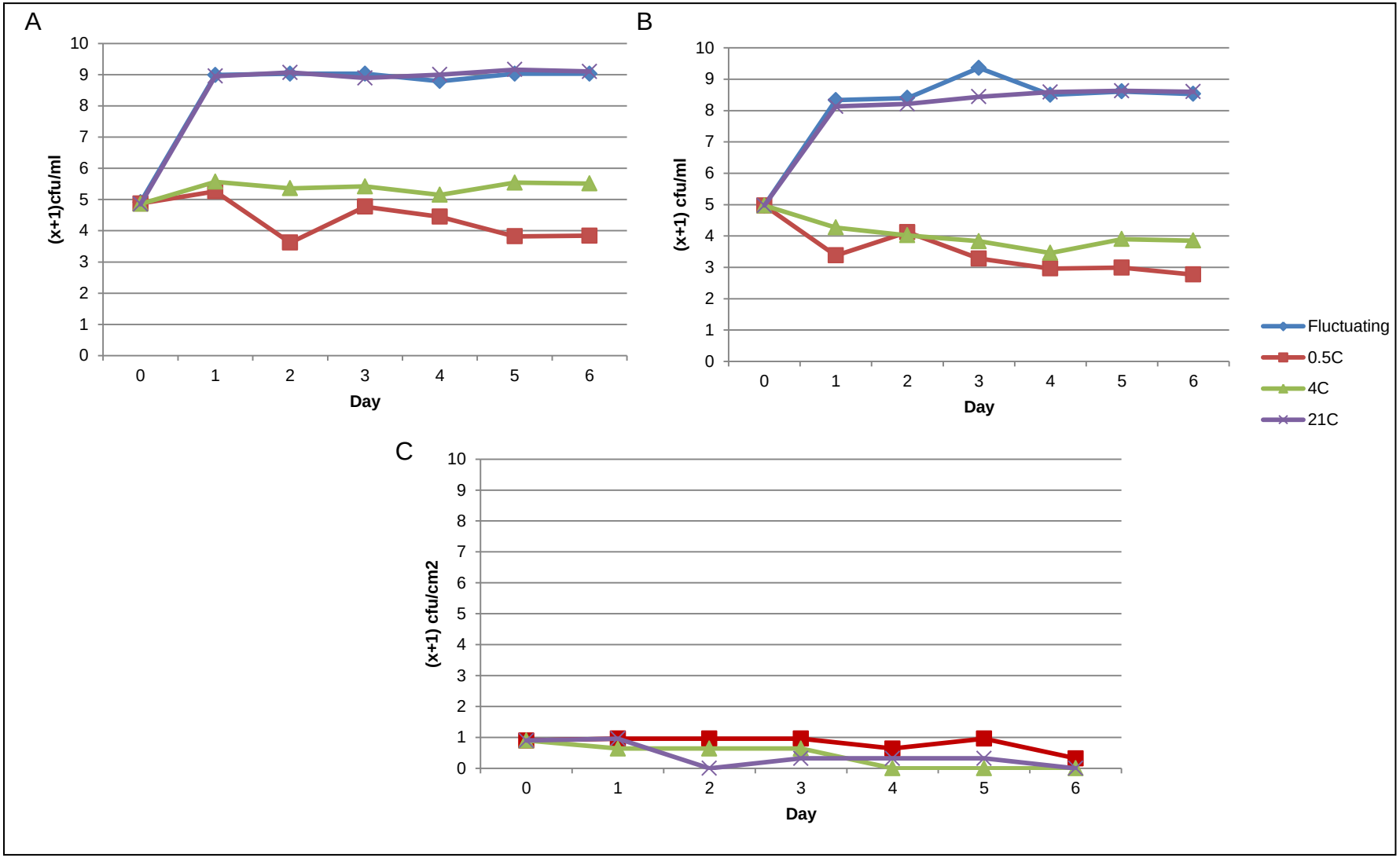

Figure 6.1 Growth dynamics of *Escherichia coli* O157:H7 under varying temperature conditions in a nutrient rich (A) and nutrient poor (B) broth and on a nutrient free tile (C).

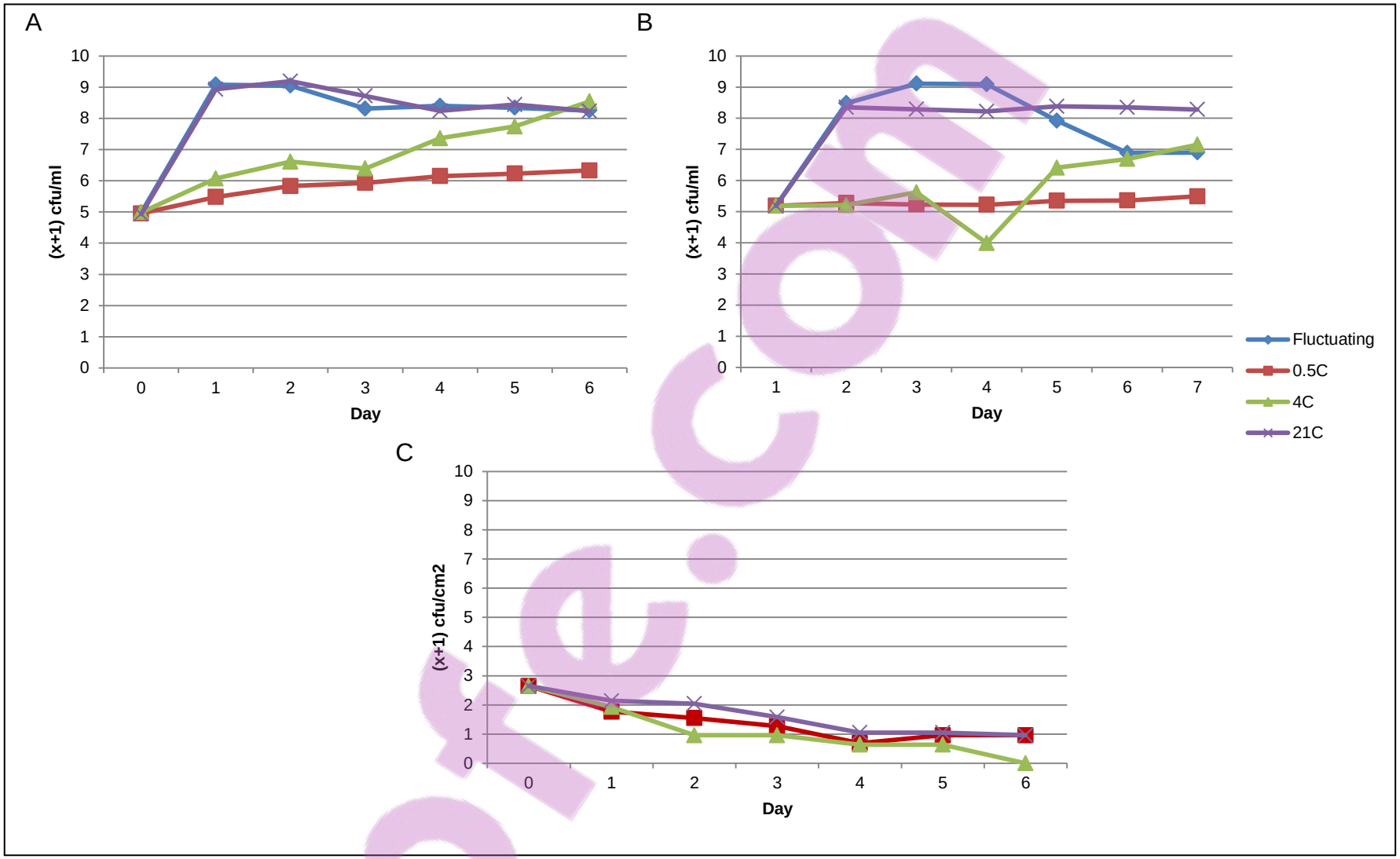

Figure 6.2: Growth dynamics of *Listeria monocytogenes* under varying temperature conditions in a nutrient rich (A) and nutrient poor (B) broth and on a nutrient free tile (C).

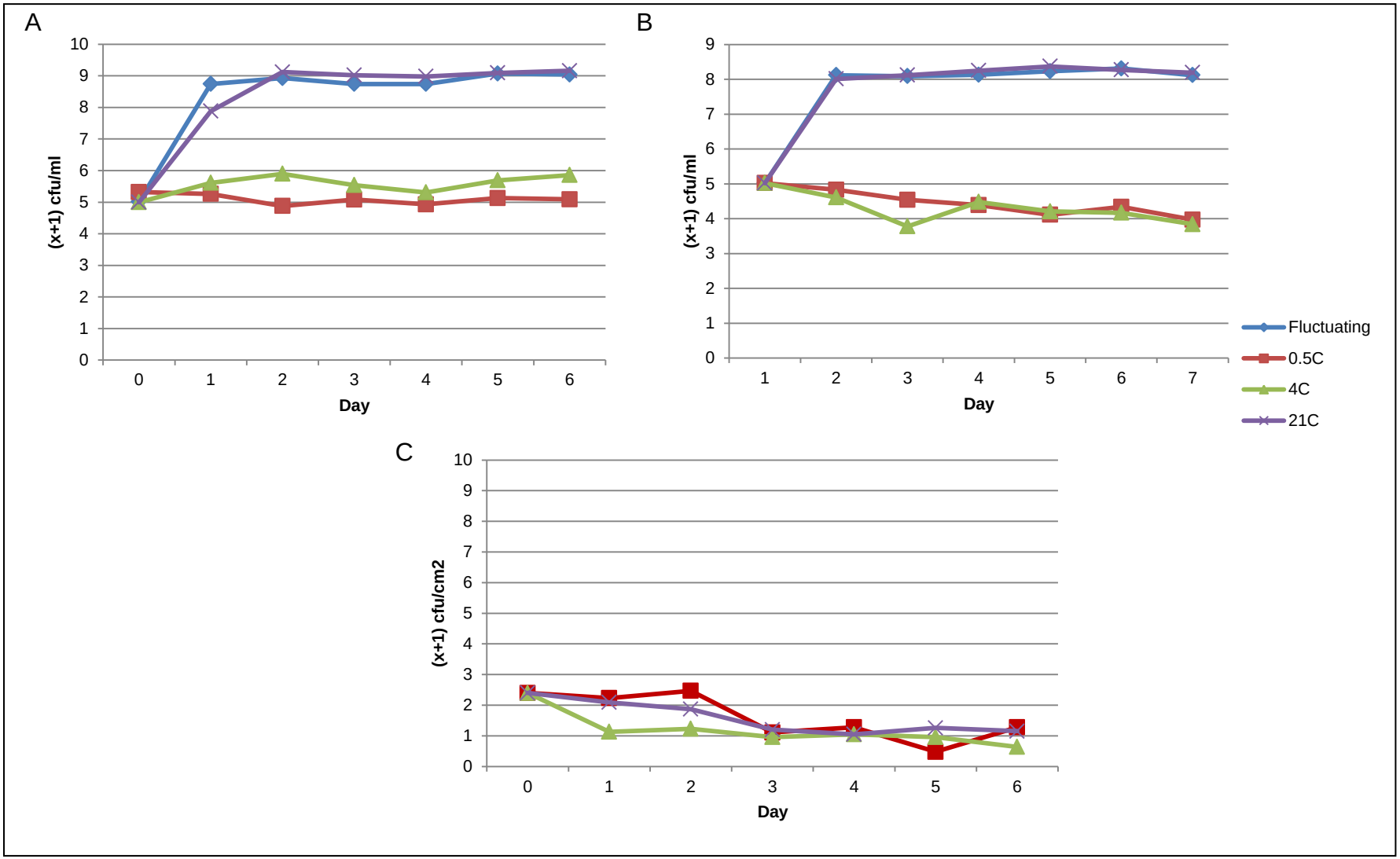

Figure 6.3: Growth dynamics of *Salmonella* Typhimurium under varying temperature conditions in a nutrient rich (A) and nutrient poor (B) broth and on a nutrient free tile (C).

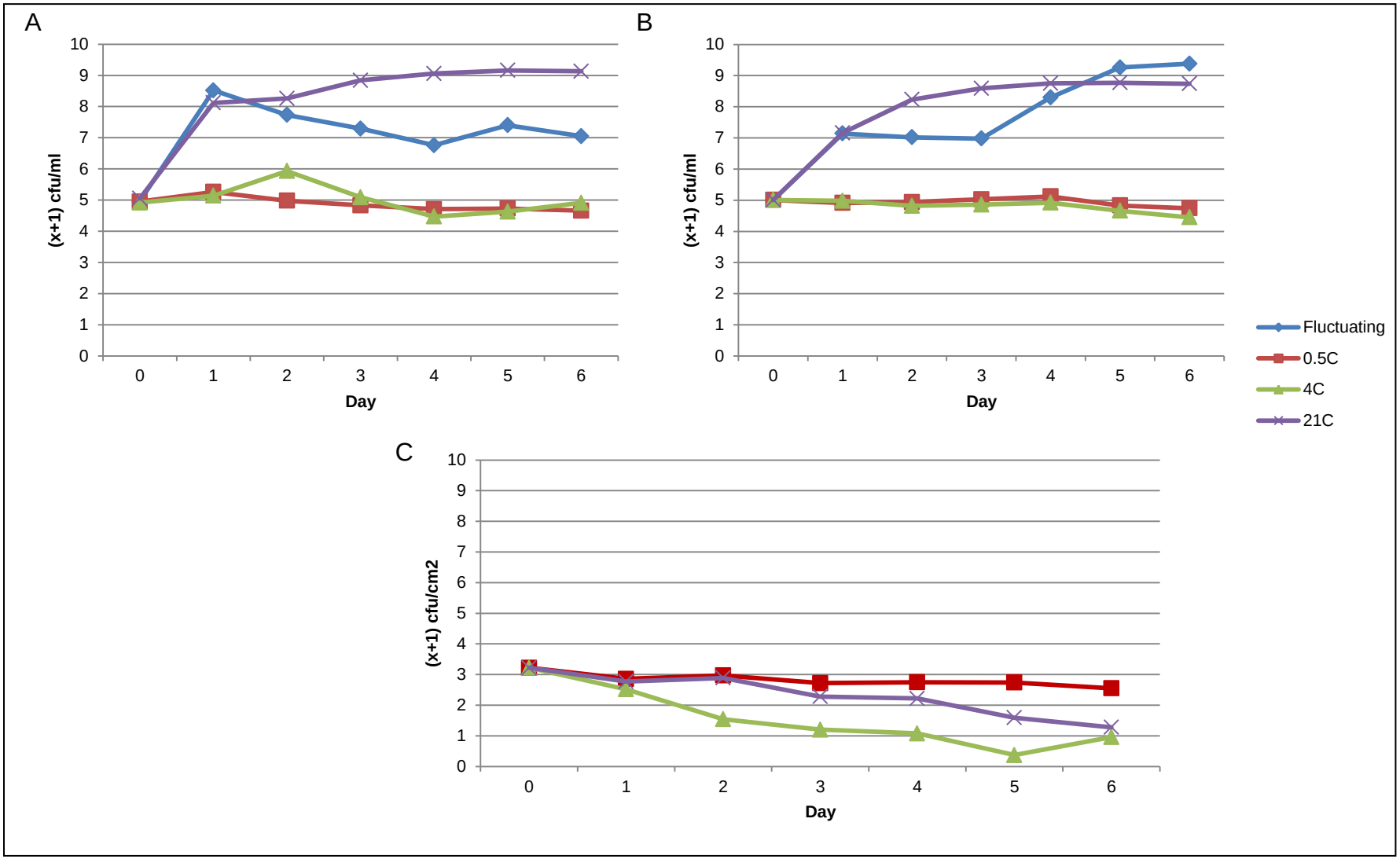

Figure 6.4: Growth dynamics of *Staphylococcus aureus* under varying temperature conditions in a nutrient rich (A) and nutrient poor (B) broth and on a nutrient free tile (C).

Escherichia coli O157:H7 when incubated at 4^{o}C was able to grow in nutrient rich conditions but not in nutrient poor conditions (Figure 6.1; Appendix 1). Titres recorded on Day 1 to Day 6 were not significantly different (Figure 6.1; Appendix 1). Day 0 and Day 4 were not significantly different (Figure 6.1; Appendix 1). But titres recorded on Day 0 were significantly lower that on Days 1, 2, 3, 5 and 6 (Figure 6.1; Appendix 1). Conversely in nutrient poor broth, *E. coli* O157:H7 exhibited the highest titres on Day 0 (Figure 6.1; Appendix 1). Titres on days 1, 2, 3, 5 and 6 were significantly lower than titres on Day 0 (Figure 6.1; Appendix 1). Titres determined on Day 4 were significantly lower than titres recorded on Day 1 (Figure 6.1; Appendix 1).

As expected *E. coli* O157:H7 was able to grow in both nutrient conditions at 21^{o}C, with both nutrient conditions exhibiting the lowest titres on Day 0 and the highest on Day 6 (Figure 6.1; Appendix 1). The only difference between nutrient conditions is that the titres reached in nutrient rich conditions were higher than those in nutrient poor conditions (Figure 6.1; Appendix 1). *Escherichia coli* O157:H7 incubated in the nutrient rich broth at 21^{o}C increased from Day 0 to Day 1, which then remained constant throughout until Day 6 (Figure 6.1; Appendix 1). Days 1, 2, 3 and 4 were not significantly different and days 1, 2, 4 and 6 were not significantly different and days 1, 2, 3 and 4 were not significantly different (Figure 6.1; Appendix 1). Similarly, *E. coli* O157:H7 growing in nutrient poor broth titres increased from Day 0 to Day 6. Titres recorded on Day 1 and Day 2 were significantly higher than titres on Day 0 (Figure 6.1; Appendix 1). Titres on Day 3 and Day 4 were significantly higher than Day 1 and Day 2 (Figure 6.1; Appendix 1). Titres on days 4, 5 and 6 were not significantly different and were the highest recorded for *E. coli* O157:H7 incubated at 21^{o}C in a nutrient poor broth (Figure 6.1; Appendix 1).

Listeria monocytogenes titres demonstrated an overall increase at all constant temperatures but it occurred faster and remained more stable at 21^{o}C, than at 0.5^{o}C and 4^{o}C (Figure 6.2; Appendix 1).

During growth in the nutrient rich broth at 0.5^{o}C, *L. monocytogenes* titres increased systematically. Titres of *L. monocytogenes* were the lowest on Day 0 and Day 1, with a subsequent increase on Day 2 (Figure 6.2; Appendix 1). Days 2, 3, 4 and 5 were not significantly different and days 3, 4, 5 and 6 were not significantly different (Figure 6.2; Appendix 1). Day 6 was however significantly higher than Day 2 (Figure 6.2; Appendix 1). Therefore there was an overall increase in *L. monocytogenes* titres from Day 0 to Day 6 in nutrient rich broth. *Listeria monocytogenes* titres were maintained in nutrient poor broth

when growing at 0.5°C, with some fluctuation in titres observed (Figure 6.2; Appendix 1). Days 0, 1, 2, 3, 4 and 5 were not significantly different, and days 1, 4, 5 and 6 were not significantly different (Figure 6.2; Appendix 1).

Listeria monocytogenes increased in titres at 4°C both in the nutrient rich and nutrient poor broths (Figure 6.2; Appendix 1). Titres of *L. monocytogenes* were the lowest on Day 0 for both nutrient conditions (Figure 6.2; Appendix 1). *Listeria monocytogenes* growth in nutrient rich broth increased significantly from Day 0 to Day 1 (Figure 6.2; Appendix 1). Titres on Day 1, Day 2 and 3 were not significantly different (Figure 6.2; Appendix 1). Titres recorded on Day 2, Day 3 and Day 4 were not significantly different and titres recorded on Day 4 and Day 5 were not significantly different (Figure 6.2; Appendix 1). Titres of *L. monocytogenes* incubated at 4°C on Day 5 and Day 6 were not significantly different (Figure 6.2; Appendix 1). An overall growth was determined for *L. monocytogenes* growing in a nutrient rich broth from Day 0 to Day 6 (Figure 6.2; Appendix 1). Following the incubation at 4°C in nutrient poor broth an overall increase was recorded for *L. monocytogenes* from Day 0 to Day 6 (Figure 6.2; Appendix 1). Titres recorded on Day 0 and Day 1 were not significantly different (Figure 6.2; Appendix 1). Titres on Day 2 were significantly higher than titres recorded on Day 0 and Day 1 (Figure 6.2; Appendix 1). Titres recorded on Day 2 were significantly higher than titres recorded on Day 3 (Figure 6.2; Appendix 1). On Day 3 the lowest titres of *L. monocytogenes* in the nutrient poor broth incubated at 4°C were recorded (Figure 6.2; Appendix 1). On Day 4 there was a significant increase in titres from Day 3 but titres on Day 4 were not significantly different to titres on Day 5 and to Day 2 (Figure 6.2; Appendix 1). Following Day 5 there was a final increase in *L. monocytogenes* titres to the highest recorded *L. monocytogenes* titre in nutrient poor broth over the six day period (Figure 6.2; Appendix 1).

At 21°C, *L. monocytogenes* titres fluctuated when incubated in the nutrient rich broth, but an overall increase was recorded (Figure 6.2; Appendix 1). The lowest *L. monocytogenes* titres for the six day trial were recorded at the time of inoculation and the highest titres on Day 1 and Day 2 (Figure 6.2; Appendix 1). On Day 3 the titres were significantly lower than on Day 2 but not significantly different from those on Day 1 (Figure 6.2; Appendix 1). Following Day 3, a significant decrease in titres was recorded on Day 4 (Figure 6.2; Appendix 1). Titres of *L. monocytogenes* achieved on Day 4 when incubated at 21°C in a nutrient rich broth were maintained until Day 6 (Figure 6.2; Appendix 1). *Listeria monocytogenes* titres increased in nutrient rich broths under all constant temperature conditions, but the increase at 21°C occurred faster than at 0.5°C and 4°C (Figure 6.2; Appendix 1). In the nutrient poor broth there was a significant increase in *L. monocytogenes* titres following incubation at 21°C from

Day 0 to Day 1 (Figure 6.2; Appendix 1). Following Day 1 all titres were maintained until Day 6 (Figure 6.2; Appendix 1).

Salmonella Typhimurium titres in a nutrient rich broth at a constant temperature of 0.5^{o}C did not significantly differ throughout the study to the titres upon inoculation (Figure 6.3; Appendix 1). In contrast *Salmonella* Typhimurium titres in a nutrient poor broth incubated at 0.5^{o}C decreased (Figure 6.3; Appendix 1). The highest titre in the study was determined on the day of inoculation (Day 0). Titres on Day 1 were not significantly different to titres on Day 0 (Figure 6.3; Appendix 1). Titres recorded on Day 1 and Day 2 were not significantly different, but titres recorded on Day 2 were significantly lower than titres on Day 0 (Figure 6.3; Appendix 1). Titres recorded on Day 2, Day 3 and Day 5 were not significantly different (Figure 6.3; Appendix 1). *Salmonella* Typhimurium titre recorded Day 4 and Day 6 were not significantly different (Figure 6.3; Appendix 1). There was an overall decrease in *Salmonella* Typhimurium titres observed when incubated at 0.5^{o}C in a nutrient poor broth from Day 0 to Day 6 (Figure 6.3; Appendix 1).

Salmonella Typhimurium was observed to grow when incubated in a nutrient rich broth at 4^{o}C, the lowest titre recorded was on Day 0 (Figure 6.3; Appendix 1). Titres recorded on Day 1 were significantly higher than titres on Day 0, titres achieved on Day 1 were maintained until Day 6 with slight fluctuations (Figure 6.3; Appendix 1). Titres recorded on Days 1, 2, 3, 5 and 6 were not significantly different and titres recorded on days 1, 3, 4 and 5 were not significantly different (Figure 6.3; Appendix 1). In a nutrient poor broth, *Salmonella* Typhimurium demonstrated an overall decrease in titre from Day 0 to Day 6. Day 0 exhibited the highest *Salmonella* Typhimurium titres in the study and the *Salmonella* Typhimurium titres on Day 1 were significantly lower than Day 0 (Figure 6.3; Appendix 1). Titres recorded on Day 1 and Day 3 were not significantly different and titres recorded on days 3, 4 and 5 were not significantly different (Figure 6.3; Appendix 1). Titres recorded on Day 5 and Day 6 were not significantly different (Figure 6.3; Appendix 1). Titres recorded for *Salmonella* Typhimurium on Day 6 was not significantly different to Day 2 (Figure 6.3; Appendix 1). Titres recorded on Day 2 and 6 were the lowest titres of all six days (Figure 6.3; Appendix 1).

Salmonella Typhimurium in a nutrient rich and nutrient poor broth is able to increase systematically over a period of 6 days at 21^{o}C (Figure 6.3; Appendix 1). In the nutrient rich broth the highest titres are achieved following 2 days, where in a nutrient poor broth the highest titres are achieved following 3 days (Figure 6.3; Appendix 1). *Salmonella* Typhimurium titres in a nutrient rich broth incubated at 21^{o}C were the lowest on the day of

inoculation (Figure 6.3; Appendix 1). Titres on Day 1 were significantly higher than titres on Day 0 (Figure 6.3; Appendix 1). Titres again significantly increased from Day 1 to Day 2 (Figure 6.3; Appendix 1). Titres recorded on Day 1 and Day 2 were not significantly different to titres recorded on days 3, 4 and 5 (Figure 6.3; Appendix 1). Titres recorded on Day 6 were not significantly different to titres recorded on days 2, 3, 4 and 5 (Figure 6.3; Appendix 1). *Salmonella* Typhimurium exhibited an overall growth in nutrient rich broth at 21^{o}C. Similarly, in a nutrient poor broth there was an overall growth pattern at 21^{o}C (Figure 6.3; Appendix 1). Titres on Day 0 were the lowest *Salmonella* Typhimurium titres in the six day trail (Figure 6.3; Appendix 1). Titres on Day 1 were significantly higher than titres recorded on Day 0 (Figure 6.3; Appendix 1). Recorded titres of *Salmonella* Typhimurium on Day 1, Day 2 and Day 6 were not significantly different (Figure 6.3; Appendix 1). Titres on Days 3, 4, 5 and 6 were not significantly different and were the highest recorded titres of *Salmonella* Typhimurium during the six day trial (Figure 6.3; Appendix 1). Titres on days 3, 5 and 6 were not significantly different (Figure 6.3; Appendix 1).

Staphylococcus aureus titres remained relatively constant when incubated in a nutrient rich broth at 0.5^{o}C, with titres recorded on Day 0 and Day 6 not significantly different (*P=0.0153)* (Figure 6.4; Appendix 1). Titres recorded on Days 1, 2 and 3 were not significantly different and were the highest recorded for *S. aureus* titres when incubated at 0.5^{o}C in nutrient rich broth (Figure 6.4; Appendix 1). Titres recorded on days 1, 3, 4 and 5 were not significantly different and titres on days 3, 4, 5 and 6 were not significantly different (Figure 6.4; Appendix 1). Similarly in a nutrient poor broth the titres remained relatively constant but there was an overall significant decrease in *S. aureus* titres (Figure 6.4; Appendix 1). Titres recorded on Days 0, 1, 2, 3 and 4 were not significantly different and were the highest recorded titres for *S. aureus* incubated at 0.5^{o}C in a nutrient poor broth (Figure 6.4; Appendix 1). Titres recorded on days 0, 1, 2, 3 and 5 were not significantly different and titres recorded on days 1, 2, 5 and 6 were not significantly different (Figure 6.4; Appendix 1). Titres recorded on Day 0 and Day 6 were significantly different and therefore there was an overall decrease in *S. aureus* titres when incubated at 0.5^{o}C in a nutrient poor broth.

When incubated at 4^{o}C in nutrient rich broth, *S. aureus* titres were not recorded to increase or decrease overall (Figure 6.4; Appendix 1). Titres on days 0, 1, 3, 4, 5 and 6 were not significantly different and titres on days 1, 2 and 3 were not significantly different (Figure 6.4; Appendix 1). When *S. aureus* is incubated in a nutrient poor broth at 4^{o}C there was an overall and systematic decrease in titres (Figure 6.4; Appendix 1). Titres on days 0, 1, 2, 3 and 4 were not significantly different but titres on days 1, 2, 3, 4, 5 and 6 were not

significantly different and titres recorded on days 5 and 6 were not significantly different (Figure 6.4; Appendix 1). Titres on Day 6 were however significantly lower than titres on days 0, 1, 2, 3 and 4, therefore demonstrating the evident decrease in titres when incubated in nutrient poor broth at 4^{o}C (Figure 6.4; Appendix 1).

At 21^{o}C, *S. aureus* titres both in the nutrient rich and poor broth all increased systematically; although in the nutrient poor broth the increase to the highest titres took more time. In the nutrient rich broth the lowest titre recorded was on Day 0. Titres recorded on Day 1 were significantly higher than titres on Day 0 (Figure 6.4; Appendix 1). Titres recorded on Day 1 were maintained until Day 2 (Figure 6.4; Appendix 1). Titres on Day 3 were significantly higher than titres on Day 2 (Figure 6.4; Appendix 1). Titres recorded on Day 3 were not significantly different to titres recorded on Day 4 or Day 6 (Figure 6.4; Appendix 1). Titres recorded on Day 4 were maintained to the completion of the study on Day 6 (Figure 6.4; Appendix 1). In the nutrient poor broth, *S. aureus* titres increased significantly from Day 0 to Day 1 (Figure 6.4; Appendix 1). Titres again increased from Day 1 to Day 2 and then titres increased again from Day 2 to Day 3 (Figure 6.4; Appendix 1). *Staphylococcus aureus* titres recorded on Day 3 were maintained until Day 6 (Figure 6.4; Appendix 1). Titres recorded on Days 3, 4, 5 and 6 were the highest *S. aureus* titres recorded of all six days (Figure 6.4; Appendix 1).

3.3. Growth dynamics of foodborne pathogens on tiles at constant temperatures

Escherichia coli O157:H7 incubated on tiles at 0.5^{o}C, 4^{o}C and 21^{o}C did not demonstrate a significant increase or decrease (Figure 6.1; Appendix 1). Titres of *E. coli* O157:H7, even though there was no significant difference from the day of inoculation, were not detected from Day 4 at 4^{o}C, nor on Day 6 at 21^{o}C but were detected from tiles on Day 6 at 0.5^{o}C (Figure 6.1; Appendix 1).

At 0.5^{o}C, *L. monocytogenes* titres recorded were the highest on Day 0 and then titres significantly decreased on Day 1 (Figure 6.2; Appendix 1). *Listeria monocytogenes* titres were not significantly different on Day 1, Day 2 and Day 3 (Figure 6.2; Appendix 1). *Listeria monocytogenes* titres recorded on Day 4 were significantly lower than titres on days 1, 2 and 3 (Figure 6.2; Appendix 1). The titre observed on Day 4 remained constant to the completion of the study (Figure 6.2; Appendix 1). *Listeria monocytogenes* titres when incubated at 0.5^{o}C were not significantly different on days 3, 5 and 6 (Figure 6.2; Appendix 1). When incubated at 4^{o}C, the *L. monocytogenes* titres decreased systematically with Day 0 having the highest

titre followed by Day 1 (Figure 6.2; Appendix 1). Titres on Day 2 were significantly higher than tires on Day 1 (Figure 6.2; Appendix 1). Titres recorded on Day 2 were not significantly different to days 3, 4 and 5 (Figure 6.2; Appendix 1). Titres recorded on Day 6 were the lowest for all six days of the experiment (Figure 6.2; Appendix 1). The titre of *L. monocytogenes* when incubated at 21^{o}C also systematically decreased throughout the 6 day study (Figure 6.2; Appendix 1). Tiles with *L. monocytogenes* incubated at 21^{o}C demonstrated highest titre at the time of inoculation (Figure 6.2; Appendix 1). Day 0 was significantly higher than Day 1 and Day 1 was not significantly different to Day 2 (Figure 6.2; Appendix 1). Titres determined on Day 3 were significantly less than titres on Day 1 and Day 2 (Figure 6.2; Appendix 1). Titres recorded on days 4, 5 and 6 were significantly less than on Day 3 (Figure 6.2; Appendix 1). *Listeria monocytogenes* survived on the nutrient free tiles at 0.5^{o}C and 21^{o}C, but not at 4^{o}C (Figure 6.2; Appendix 1).

Salmonella Typhimurium titres decreased when incubated at all three temperatures on the tile surface but were detected on Day 6 at all three temperatures, therefore demonstrating the ability of the organism to survive even with the lack of nutrients (Figure 6.3; Appendix 1). Titres on Day 0 were the highest for all three temperatures (Figure 6.3; Appendix 1). *Salmonella* Typhimurium titres were maintained at the same level as Day 0 until Day 2 when incubated at 0.5^{o}C or 21 ^{o}C (Figure 6.3; Appendix 1). Titres on Day 3 were significantly lower than titres on Day 2 (Figure 6.3; Appendix 1). *Salmonella* Typhimurium titres on Day 3 were maintained until Day 6 when incubated at 0.5^{o}C or 21^{o}C (Figure 6.3; Appendix 1). At 4^{o}C, *Salmonella* Typhimurium titres decreased significantly on Day 1 when compared to Day 0 (Figure 6.3; Appendix 1). Titres recorded on Day 1 were maintained until Day 5 (Figure 6.3; Appendix 1). Titres recorded on Day 3 were not significantly different to days 4, 5 and (Figure 6.3; Appendix 1).

An overall decrease in *S. aureus* titres was observed on a nutrient free tile at all three temperatures and *S. aureus* was still detected on tiles from all three temperatures on Day 6 (Figure 6.4; Appendix 1). When incubated at 0.5^{o}C the highest titre was recorded on Day 0, followed by a significant decrease on Day 1 which maintained to Day 2 (Figure 6.4; Appendix 1). Titres recorded on Day 3 were significantly lower than titres on Day 2 (Figure 6.4; Appendix 1). Titres recorded on Day 3 were maintained until Day 6 (Figure 6.4; Appendix 1). Titres on days 1, 3, 4 and 5 were also not significantly different (Figure 6.4; Appendix 1). Interestingly, *S. aureus* titres on tiles following incubation at 0.5^{o}C at the end of the study were higher than those on Day 6 incubated at 4^{o}C or 21^{o}C (Figure 6.4; Appendix 1). A significant decrease in *S. aureus* titres were observed following Day 1 of 4^{o}C incubation. A

significant decrease was recorded in titres from Day 1 to Day 2 (Figure 6.4; Appendix 1). Titres recorded on Day 2 were maintained until Day 3 (Figure 6.4; Appendix 1). Titres recorded for Day 3 and Day 4 were not significantly different, however, titres recorded on Day 2 and Day 4 were significantly different (Figure 6.4; Appendix 1). Titres recorded on Day 5 were significantly lower than titres recorded for Day 4 (Figure 6.4; Appendix 1). Titres recorded on Day 6 were significantly higher than titres recorded on Day 5 (Figure 6.4; Appendix 1). At 21^{o}C *S. aureus* titres on the nutrient free tiles followed a more systematic decrease (Figure 6.4; Appendix 1). The highest *S. aureus* titre was recorded on Day 0 (Figure 6.4; Appendix 1). Titres recorded on Day 1 were significantly lower than titres recorded on Day 0 (Figure 6.4; Appendix 1) Titres recorded on Day 1 were maintained until Day 2 (Figure 6.4; Appendix 1). Titres recorded on Day 3 were significantly lower than titres on Day 2 and these titres were maintained until Day 4 (Figure 6.4; Appendix 1). Titres recorded on Day 5 were significantly lower than titres on Day 4 (Figure 6.4; Appendix 1). Titres recorded on Day 6 were significantly lower than titres on Day 5 (Figure 6.4; Appendix 1).

3.4. Growth dynamics of foodborne pathogens on fruit at fluctuating temperatures

All pathogens were able to survive on peaches and plums from Day 0 to Day 6 under fluctuating temperature regimes (Table 6.1).

No significant difference in *S. aureus* titres was observed on peaches neither was there a significant difference in *E. coli* O157:H7 titres on peaches, except following 0.5^{o}C where titres decreased significantly (Table 6.1). *Listeria monocytogenes* had the highest titres on peaches on day 0. Titres of *L. monocytogenes* only decreased significantly after day 4 and remained constant for the duration of the study (Table 6.1). *Salmonella* Typhimurium titres on peaches were significantly lower for day 0 and 1 until after the -0.5^{o}C and 21^{o}C storage where titres were seen to increase and were then maintained at the same level as day 0, day 1 and day 4 (Table 6.1).

Escherichia coli O157:H7 titres on plums were the highest on day 0 followed by a significant decrease on Day 1, followed by a further significant decrease on Day 4 after which titres remained constant (Table 6.1). *Staphylococcus aureus* and *Salmonella* Typhimurium also followed the same trend on plums with a significant decrease following Day 1, with stabilisation in titres from days 4 to 6 (Table 6.1). *Listeria monocytogenes* titres increased significantly following Day 0 with a significant decrease to Day 4 which then remained constant until Day 6 (Table 6.1).

Table 6.1: Summary of the pathogen titres on stone fruit following artificial inoculation and simulating the cold chain

Fruit	Day	cfu/cm^2			
		Escherichia coli	***Listeria monocytogenes***	***Salmonella* Typhimurium**	***Staphylococcus aureus***
Peach Fruit	**Day 0**	3.21 **a** [x]	3.74 **a**	1.43 **b**	4.55 **a**
	Day 1	2.02 **b**	3.16 **ab**	0.88 **b**	4.19 **a**
	Day 4	4.12 **a**	2.83 **b**	2.74 **a**	4.15 **a**
	Day 6	3.43 **a**	3.48 **ab**	1.59 **ab**	4.38 **a**
	LSD [y]	1.1271	0.6506	1.1989	0.5314
	***P* value** [z]	0.0063	0.0441	0.0273	0.3999
Plum Fruit	**Day 0**	2.88 **a**	3.23 **b**	2.40 **a**	4.73 **a**
	Day 1	2.01 **b**	4.19 **a**	2.81 **a**	4.97 **a**
	Day 4	0.11 **c**	0.80 **c**	0.38 **b**	3.90 **b**
	Day 6	0.39 **c**	1.18 **c**	0.53 **b**	3.87 **b**
	LSD	0.8264	0.7922	<0.0001	0.5999
	***P* value**	<0.0001	<0.0001	0.7248	0.0008

[x]: Values followed by the same **bolded letter** means that the two values are not significantly different according to the Fischer Test (P<0.05); [y]: LSD is the least significant difference within one temperature condition; [z]: *P* value is significant if the value is less than 0.05.

4. Discussion

All four pathogens exhibited an overall increase in titres when grown in a nutrient –rich or –poor broth at fluctuating temperatures. Therefore it can be concluded that available nutrients do not play a role in the growth of these pathogens under such temperature conditions. However, on peaches the titre of all four pathogens remained constant compared to the initial viable concentration, while on plums an overall decrease was noted. Temperature alone is therefore not the limiting factor in the growth of these organisms. Other factors that can influence the growth of these four pathogens is the pH and water activity of the substrate (Adams and Moss, 2000). Plums are more acidic (pH 2.8-3.0) than peaches (pH 3.4 to 4.1). *Escherichia coli* O157:H7 have different behaviour patterns depending on the temperature, pH and water activity (Rocelle *et al.,* 1996). Rocelle *et al.* (1996) determined that at pH 4.8, *E. coli* O157:H7 was not able to grow as vigorously as at pH 5.4 and 6.0 at 4, 20 and 30^{o}C. Therefore with an increase in acidity, *E. coli* O157:H7's growth decreases. Audia *et al.* (2001) reported however that *E. coli* O157:H7 were able to tolerate pHs as low as 2. The authors also demonstrated that water activity only had a major effect on the growth of *E. coli* O157:H7 at 30^{o}C (Rocelle *et al.,* 1996), therefore higher temperatures than used in this

study. The pH of the substrate (plums and peaches) therefore has an important role in the growth of *E. coli* O157:H7. Tienungoon *et al.* (2000) determined that *L. monocytogenes* is able to grow at a pH of 6 at 5 ^{o}C and 20^{o}C but no growth was recorded when the pH was decreased to 4. Similarly in this study there was no growth on peaches (pH 3.4 to 4.1) and a decrease in titre on plums (pH 2.8-3.0) due to the different pH levels. Again for *Salmonella* Typhimurium incubated under fluctuation temperatures, this was not the limiting factor as growth was observed in the broths but not on the peaches or plums. Gordon and Small (1993) demonstrated that *Salmonella* Typhimurium was unable to survive below a pH of 3. Waterman and Small (1998) demonstrated that the acidity of a substance is a critical factor in determining the survival of enteric pathogens. *Staphylococcus aureus* were unable to grow or survive at a pH of 3 at 5 or 20^{o}C for longer than one day and at a pH of 4, *S. aureus* was only able to survive for approximately 2.5 days (Whiting *et al*., 1996). For all four foodborne pathogens the acidity of the peaches and plums were the limiting factor of growth because at a pH of 7 under both nutrient conditions all four pathogens grew.

According to the authors' knowledge, this is the first study comparing the growth of *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium and *S. aureus* at 0.5^{o}C in different media/environments. It was found that *L. monocytogenes* was the only one of the four foodborne pathogens tested able to grow at 0.5^{o}C. *Listeria monocytogenes*, a psychrotroph, is able to grow from 2^{o}C and can survive freezing conditions (-18^{o}C). This study demonstrates that *L. monocytogenes* is able to grow at 0.5^{o}C irrespective of the amount of nutrients present when at a neutral pH and optimum water activity. When no nutrients or water are present, such as on a floor tile environment, *L. monocytogenes* is not able to grow at these low temperatures and the combined effect of all three limiting factors (temperature, nutrient content and water activity) play a synergistic role in suppressing growth and causing a decrease in titres. *Salmonella* Typhimurium and *S. aureus* only exhibited an overall decrease in titres when the nutrients were not present at a high concentration, when nutrients were limited or not present there was an overall decrease in titre when incubated at 0.5^{o}C. Neither *S. aureus* nor *Salmonella* Typhimurium are psychrotrophic organisms. Therefore when nutrients, pH and water activity are optimum *Salmonella* Typhimurium and *S. aureus* are able to survive without decrease at 0.5^{o}C, but if the nutrients are lowered then there is a decrease in pathogen titre. *Escherichia coli* O157:H7 titres decreases at 0.5^{o}C irrelevant of the nutrient concentration.

Rocelle *et al.* (1996) demonstrated that *E. coli* O157:H7 was not able to substantially grow at 5^{o}C following two days, in the present study this was the case for the nutrient poor broth but

there was a 1 log increase in titre following two days incubation at 4°C in a nutrient rich broth. Overall *E. coli* O157:H7 and *Salmonella* Typhimurium incubated at 4°C showed a general increase in growth in a nutrient rich broth and a decrease in a nutrient poor broth, therefore the combination of the low temperature and the reduced nutrients synergistically limit the growth of *E. coli* O157:H7 and *Salmonella* Typhimurium. Some *Salmonella* strains have been shown to grow at 4°C, however growth is slower than at an optimum temperature (Russell and Gould, 2002 as cited by Russell, 2002). Mattick *et al.* (2003) also demonstrated that *Salmonella* Typhimurium is able to grow at 8°C. Pintar *et al.* (2007) however demonstrated that *Salmonella* Typhimurium was unable to grow, but sustained the original titre, on chicken breast with the addition of a nutrient broth. As with incubation at 0.5°C, *L. monocytogenes* is able to grow when in a nutrient rich broth but also in a nutrient poor broth. Tienungoon *et al.* (2000) demonstrated that *L. monocytogenes* was able to grow at 4°C at a pH of 6. *Listeria monocytogenes* at 4°C is not limited by nutrients because the organism can grow in a range from 2°C. Some strains of *S. aureus* have also been recorded to grow at refrigeration temperatures (Russell and Gould, 2002 as cited by Russell, 2002). In this study *S. aureus* was unable to grow in the nutrient rich broth and titres decreased with limited nutrients at less than 4 °C. Whiting *et al.* (1996), demonstrated that *S. aureus* had a D_4 of 2500 hours, therefore following approximately 100 days 10% of the initial inoculum was still present when incubated at 4°C.

As expected all four pathogens were able to grow at 21°C within a nutrient –rich or –poor broth, irrespective of the level of available nutrients. The findings of this study are in agreement with previous studies on the growth of *E. coli* O157:H7 (Rocelle *et al*., 1996), *L. monocytogenes* (Tienungoon *et al.,* 2000), *Salmonella* Typhimurium (D'Auost, 1991) and *S. aureus* (Whiting *et al.,* 1996) at an optimum temperature of 21°C when nutrients are available.

Even though these pathogens were able to grow in liquid broth containing nutrients within their optimum range of temperature, these organisms were not able to grow on a nutrient free tile surface. All the pathogens were able to survive on the surface with an overall decreasing trend except *E. coli* O157:H7 that was unable to be detected following six days at 4 or 21°C nor was *L. monocytogenes* at 4°C. Kusumaningrum *et al.* (2003) made the same observation that *S. aureus* and *Salmonella* Enteritidis exhibited a significant decrease in titres following 96 hours (or 4 days) at room temperature on a dry stainless steel surface to only just detectable when the initial inoculums was similar to this study. The same trend was observed when *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus*

were inoculated onto the nutrient free tiles and stored at 0.5^{o}C, 4^{o}C and room temperature (21^{o}C), indicating that the temperature was not the major factor in limiting growth but the lack of nutrients and water were synergistically limiting the growth of these pathogens. Kusumaningrum *et al.* (2003), also recorded that with the introduction of liquid food residues which resulted in an increase in nutrients and water available the survival of the pathogens increased. It is important to note that on the tiled surface all the pathogens were able to survive following 6 days, except *E. coli* O157:H7 at 4 or 21^{o}C and *L. monocytogenes* at 4^{o}C. Pathogens that were able to survive could therefore lead to cross-contamination of food products stored or moving through the specific temperatures.

In conclusion, a number of factors influence the growth or survival of foodborne pathogens on contact surfaces and on food products. This study shows that if *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium and *S. aureus* contaminate peaches or plums at a high dosage, these pathogens have the ability to survive on the fruit. The decrease in titre under cold storage conditions cannot be attributed to the limiting factor of temperature only, but possibly the synergistic effect of temperature, pH and water activity that all influence the growth of these pathogens on a fruit surface. Further research should focus on determining the synergistic effect of these factors on the growth of foodborne pathogens under cold storage conditions. On tiles, the tested pathogens are able to survive at temperatures that simulate working environments and therefore could potentially lead to the contamination of food products coming into contact with such surfaces.

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Chapter 7

Attachment and colonisation of *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* to stone fruit surfaces and survival through a simulated commercial export chain

Stacey Collignon and Lise Korsten

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Abstract

The ability of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* to attach, colonise and survive on stone fruit surface was investigated. Fifty microliters of bacterial suspension was spot-inoculated onto the sterile intact fructoplane of whole peaches and plums. Minimum time required for initial adhesion and attachment was investigated using different pathogen surface contact times. Surface colonisation patterns of the four foodborne pathogens and survival under simulated commercial export conditions were studied. *Listeria monocytogenes* and *Salmonella* Typhimurium attached immediately to stone fruit surfaces. *Escherichia coli* O157:H7 was visibly attached after only 30s and *S. aureus* after 1h direct exposure. Placing freshly harvested stone fruit under 0.5^{o}C simulated cold storage conditions significantly lowered the titre of *E. coli* O157:H7 on plums and *L. monocytogenes* and *Salmonella* Typhimurium on stone fruit. *Escherichia coli* O157:H7 and *L. monocytogenes* at a low inoculum concentration level and *S. aureus* and *Salmonella* Typhimurium at high and low concentrations did not survive the simulated export chain conditions at titres that exceeded the minimum infectious dose. *Escherichia coli* O157:H7 and *L. monocytogenes* however, were able to survive on stone fruit surfaces when inoculated with an artificially high inoculum concentration. In this case, the final titre at the end of the supply chain was above the infectious dose. This study further showed that *E. coli*

O157:H7*, L. monocytogenes, Salmonella* Typhimurium and *S. aureus* when tested at potential natural contamination levels under laboratory conditions were unable to survive simulated export conditions.

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1. Introduction

Globally more human disease outbreaks associated with foodborne pathogens have been reported which have been shown to be linked to increasing consumption of contaminated fresh produce (Todd, 1997). In addition, the increase in reported disease outbreaks can be attributed to a number of reasons, one being the shifting focus towards a healthier lifestyle and diet in more developed countries. The increased demand for year round availability of fresh fruit and vegetables and more exotic produce that are often procured from less developed countries with less effectively regulated food control systems. These global procurement patterns have resulted in more extensive supply chains, ultimately involving more complex distribution networks and longer road and sea transportation systems. More complex distribution systems in turn result in increased handling. Foodborne pathogens are transmitted through the supply chain by various vectors. Some of the more frequently reported foodborne pathogens associated with the consumption of contaminated fresh produce are *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. (CDC, 2008) and therefore should be considered in any food safety management system. *Staphylococcus aureus* has also been linked to foodborne outbreaks throughout the world. Foodborne outbreaks of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp that occurred previously were associated with the consumption of contaminated cantaloupe, cut fruit, strawberries, raspberries, tomatoes, spinach, lettuce and various other fresh produce have been well documented (Brandl and Mandrell, 2002; CDC, 2005; CDC, 2006a; CDC, 2006b; CDC, 2007; CDC, 2008; Herwaldt *et al.*, 1994; Korsager *et al*., 2005; Le Guyader *et al.*, 2004 and Seymour and Appelton, 2001). *Escherichia coli* and *Salmonella* spp. have been previously detected on the surface of stone fruit after the random sampling of fruit (Abdelnoor *et al.*, 1983).

Microbial contamination of fresh produce can occur within the pre- and post- harvest environments. The exposure of fresh produce to contaminated water, handlers or contact surfaces (Brackett, 1999; James, 2006; Rajkowski and Baldwin, 2003) increases the likelihood that foodborne pathogens can successfully attach to the fructoplane. Contamination should therefore be avoided using pre- and post- harvest production and distribution practices that prevent contamination. Fresh produce that are traded through extensive supply chains are also exposed to several possible contamination points once

leaving the farm gate. Contamination could therefore also potentially occur at any point from the farm and packhouse up to the point of handling and consumption within the importing country (Korsten and Zagory, 2006).

Prevailing environmental conditions while the fresh product is in transit is therefore important since it can potentially support microbial growth, survival or result in death of the organism thereby reducing or increasing the risk. Therefore, more stringent control at the point of production and dispatch is required to ensure that foodborne pathogens are not introduced into the food chain. In case of potential contamination, intervention strategies should be followed to ensure that the organism cannot survive or proliferate up to the point of consumption. Under effective cold chain management systems proliferation of foodborne pathogens on fresh produce surfaces can be prevented. *Escherichia coli*, *L. monocytogenes*, *Salmonella* spp. and *S. aureus* are able to survive refrigeration temperatures, with *L. monocytogenes* being able to proliferate (Baird-Parker, 2000; D'Auost, 2000; Farber and Peterkin, 2000 and Willshaw *et al.*, 2000), therefore potentially allowing survival.

Adherence, attachment, colonisation and survival of foodborne pathogens on raw fresh produce, is a critical aspect in the fruit contamination cycle (Kroupitski *et al.*, 2009). Understanding the stages of the organisms' contamination cycle will allow for the establishment of better prevention strategies within the pre- and post- harvest environment.

The aim of this study was therefore to acquire a better understanding of the potential of *E. coli* O157:H7, *L. monocytogenes*, *S. enterica* subsp. *enterica* serovar Typhimurium and *S. aureus* to adhere, attach, colonise and survive on stone fruit. In this study time-temperature exposure intervals that simulate harvesting, packing, transport, cold storage and export conditions used to retain fruit quality, control decay and extend shelf life was used to determine the likelihood of foodborne pathogen's survival on stone fruit surfaces.

2. Materials and Methods

2.1. Cultures

American Type Culture Collection cultures namely *E. coli* O157:H7 (ATCC 35150), *L. monocytogenes* (ATCC 19115), *S. enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028) and *Staphylococcus aureus* (ATCC 12600) were used as reference cultures in this study. All cultures were maintained lyophilised and stored at -70^{o}C with subcultures on

standard 1 medium (Merck) prepared 24h prior to use. Cultures were used to inoculate five replicates of 100 ml TSB (Merck) for each pathogen and were subsequently incubated at 37^{o}C for 18h to achieve a concentration of 8 log cfu/ml. Cultures were centrifuged at 5000 rpm and washed twice with sterile distilled water and finally re-suspended into 1% (w/v) Peptone Buffered Water (Merck). Cultures were subsequently serially diluted to obtain a high inoculum concentration of 7 log, and a low inoculum concentration, 5 log cfu/ml. Concentrations were confirmed by serial dilution and subsequent plating in duplicate.

2.2. Fruit

Peaches (*Prunus persica* cv. Excellence) and plums (*Prunus domestica* cv. Flavour King) were aseptically hand harvested at optimum maturity from two commercial farms in the North-West Province and Limpopo Province, respectively. The full experiment was repeated on two separate occasions. Fruit of a uniform size and weight and without pest, disease or damage were used in this study. Harvested fruit were individually bagged in paper bags and transported to the laboratory in cooler boxes and stored at 4^{o}C overnight (approx. 12 to 15 h). Collected fruit were divided into three sets. *Set one*, was used for scanning electron microscopy (SEM) analysis and contained 22 peaches (seven for each pathogen and one negative control) and 37 plums (nine fruit for each pathogen and one negative control). *Set two*, used to quantify the pathogen titre following high concentration inoculation, consisted of 50 peaches (five replicates for nine time intervals selected and five negative controls) and 62 plums (five replicates for 11 time intervals and seven negative controls). *Set three*, used to quantify the pathogen titre following low concentration inoculation, contained 30 peaches (five replicates for five day intervals and five negative controls) and 42 plums (five replicates for seven day intervals and seven negative controls). *Set one* fruit for SEM studies were thus surface sterilised using a 30 second 70% ethanol (Spurr, 1979) dip treatment followed by air drying. Fruit from *Set two* and *three* were washed using 0.05% (v/v) Sodium hypochlorite for 30s, rinsed twice with sterile distilled water and allowed to air dry.

2.3. Spot inoculation

Spot inoculation for SEM studies were done on a (5mmx5mm) surface area of the fruit marked with a felt pen and using 50µl of prepared culture per pathogen per short time intervals (0s, 30s, 60s, 1h) to determine attachment, and longer time intervals for peaches (1d, 14d, 20d and 21d as illustrated in Figure 7.1) and for plums (1d, 6d, 13d, 18d, 25d and 26d, as illustrated in Figure 7.1) using the high inoculum concentration (7 log cfu/ml). During short time intervals the culture was put directly onto the fruit and aspirated following the

respective time intervals while being kept at room temperature. The inoculated fruit surface area was subsequently rinsed by dispensing 100µl sterile distilled water onto the inoculated section, followed by aspiration and discarding the water, the rinsing process was repeated. The blocks were then aseptically excised and were immediately processed for SEM (*Set one*). Spot inoculation, using 50µl of culture, for the quantification of pathogen titres was carried out on five *Set two* (high inoculum) and five *Set three* (low inoculum) fruit per short time interval (0s, 30s, 60s, 1h, 2h) and longer day time intervals (Figure 7.1). Spot inoculation was carried out ensuring that cultures were not mixed. Following spot inoculation the final concentration of each culture on the fruit was confirmed to be 5 log (high) and 3 log (low) cfu/fruit with serial dilutions as described before. The viable *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* titres remaining on these fruit following various short and longer time intervals (0s, 30s, 60s, 1h, 2h and Figure 7.1) was determined. Once inoculated, fruit were divided according to replicates (5 each for pathogen titre determination and four each for SEM) and were distributed equally into five containers in five areas of the incubation space to allow for temperature variation within the incubation chamber.

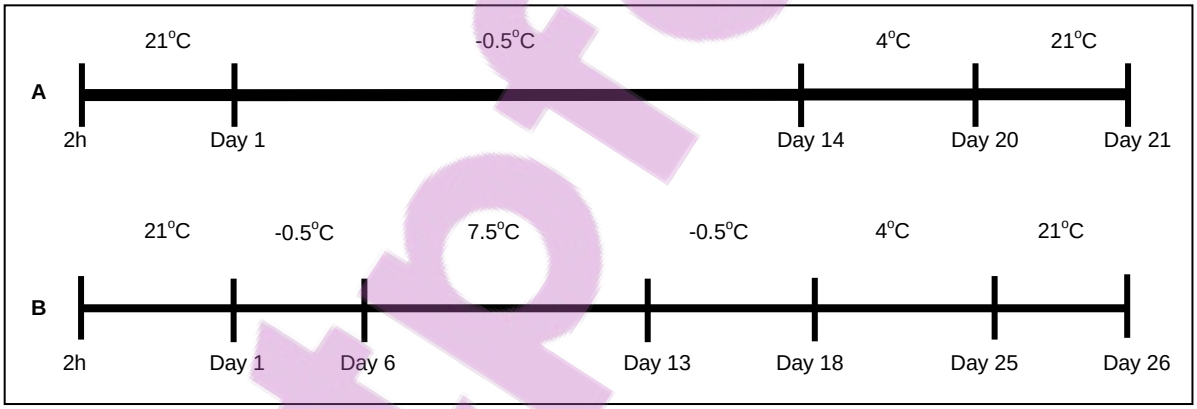

Figure 7.1: Time regime for the ship freight export for stone fruit.

Peaches (A) and plums (B).

2.4. Methodology for quantification of microorganisms

Inoculated *Set two* (high inoculum) and *Set three* (low inoculum) fruit (five replicates) were used to quantify pathogen titres on fruit after various short and longer time intervals (0s, 30s, 60s, 1h, 2h and Figure 7.1). Fruit were removed from the incubation cold storage area at the different time intervals (0s, 30s, 60s, 1h, 2h and Figure 7.1) and washed to determine the bacterial titre present on the fruit. Fruit were washed in 500ml quarter strength Ringer's solution amended with 0.02% Tween-80 (Sigma) in the Ultrasonic Bath (Labotec) for 30s.

The Ringer's solution was subsequently filtered through a 0.45nm nitrocellulose membrane. The membrane was subsequently used for serial dilution and plating in duplicate onto selective agar specific for the four pathogens [Baird-Parker medium for *S. aureus*, Oxford Listeria Selective Agar for *L. monocytogenes*, Levine Eosine-Methyl Blue Agar for *E. coli* O157:H7 and XLD Agar (all supplied by Merck) for *Salmonella* Typhimurium] was performed and counts recorded. Volume displaced (vd) for each fruit was recorded and converted to cm^2 using the following equation:

$$A=4.84[(vd)^{1/3}]^2$$ (De Jager, 1999).

Counts were converted to cfu/cm^2 and transformed to log (x+1) cfu/cm^2.

2.5. Scanning electron microscopy evaluation

Set one fruit inoculated for SEM evaluation were used to excise the marked and inoculated section of fruit. The uninoculated fruit served as negative controls. Excised sections were stored in 1ml fixing solution, containing 1ml of 25% gluteraldehyde in 0.075M phosphate buffer according to Coetzee and Van der Merwe (1994), with a modification of 25% formaldehyde. Samples were stored for a maximum of one month. Samples were rinsed three times in 0.075M phosphate buffer for 15 min each, followed by successive 15 min dehydration in 50, 70 and 90% ethanol and finally three times in 100% ethanol. Samples were critically point dried in a Bio-Rad dryer (Bio-Rad Polaron Division, England) under liquid carbon dioxide. Following drying, samples were mounted using non-conductive tape and coated for 2.5 min with 10mÅ of gold-palladium (Polaron Equipment Ltd., England) and examined under a JEOL (JSM-840) Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan) operating at 5 or 8kV. Negative controls were viewed first to become familiar with the fructoplane, followed by viewing of the higher titre and later time intervals (21 or 26d) to determine the orientation and size of the bacterial pathogens. The viewing strategy was then to view systematically through all samples from the longest exposure times (highest titres) to the shortest exposure times (lowest titres). Cells were counted (c=number of cells counted) on 15 randomly selected areas (a=area of the SEM viewing area) per sample at 3000x magnification. The following equation was generated to calculate the number of cells per centimetre squared fruit with an average of 111.15cm^2 (y) per fruit as determined in the present study (described previously).

$$Cells/cm^2 = (c)(a/y)^{-1}$$

Observations were made on 15 viewing areas per sample on each stub to determine adhesion, attachment, replication, colonisation and survival of the organisms on the fruit surfaces. Observations were subsequently calculated into a percentage of observation per

sample viewed (here forth called the rate). The attachment rate is the percentage of observed attachment and the replication rate is the percentage of observed multiplication.

2.6. Statistical Analysis

All experiments were repeated. Results obtained for each repeat were analysed together, therefore 10 replicates were analysed. Statistical analysis was performed on log cfu/cm^2 and log cells/cm^2. Data were analysed using SAS 9.2 for Windows (SAS Institute Inc., Cary, United States of America). A one-way analysis of variance was used to determine the difference in pathogen titres present on fruit surfaces. Means were analysed using the least significant difference (using the Fischer test) at a 5% level of significance.

3. Results

3.1. Surface Characteristics

The uninoculated control samples viewed reflected the fruit surface characteristics, *i.e.* trichomes, lenticels and wax structures and epidermal corrugation. It was noted, following viewing of 420 viewing areas, that the peach surfaces was extensively covered with trichomes (Figure 7.2A). The observed incidence of trichomes on this specific cultivar was high, making viewing of surface characteristics difficult. The peach surface did not appear to have many observable lenticels. The plum surface appeared smooth not very corrugated with observable lenticels and smooth wax plates (Figure 7.2B) after viewing 540 viewing areas. No microorganisms could be observed under the SEM on the control fructoplane indicating that the surface sterilisation process, using ethanol, was successful due to the toxic activity on resident microflora (Spurr, 1979). Morphological characteristics were observed under the high inoculation concentration and were all consistent for all bacterial pathogens when compared to the pathogens viewed at the longest exposure time period.

All four pathogens preferentially attached to the trichomes on peaches (Figure 7.3, 7.5, 7.7), it was observed that these pathogens were also evenly distributed over the trichome. On plums, all pathogens were able to attach to the smooth surface of the fruit but preferentially attached to areas nearby lenticel sites.

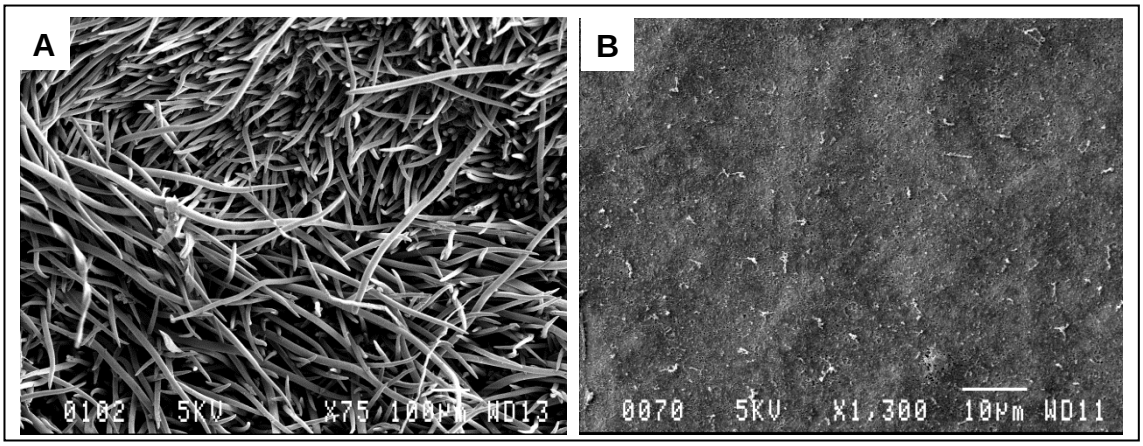

Figure 7.2: Electron Micrographs of surface of stone fruit.

Trichomes on the surface of peaches (x75 magnification) (A). The smooth surface of plums (x1300 magnification) (B).

3.2. Initial Adhesion

Initial adhesion was determined to occur at the first time interval where microbial counts (cfu/cm^2) of the high and low concentration inoculum were attained as well as when cells (not attached with polysaccharides), were first observed on the fruit under the SEM. Adherence of *Salmonella* Typhimurium (Figure 7.3C and 7.4C) and *S. aureus* (Figure 7.3D and 7.4D) occurred immediately following inoculation on stone fruit, whereas with peaches, specifically *E. coli* O157:H7, (Figure 7.3A) adhered within 30s and *L. monocytogenes* (Figure 7.3B) within 60s (Table 7.1). In both pathogen cases, adherence to plums were immediate (Figure 7.4A and 7.4B) (Table 7.1). High cell numbers of *S. aureus* were observed immediately after inoculation using the SEM and following 2h post inoculation. The colony counts were high on both peaches and plums therefore demonstrating *S. aureus*' ability to adhere to stone fruit when inoculated using both high and low concentration inoculum (Table 7.2 and Table 7.3). All four test organisms were able to adhere to stone fruit at the low concentration inoculum load with varying viable counts initially obtained (Table 7.2 and Table 7.3).

Table 7.1: Shortest time required for adhesion and attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Staphylococcus aureus* of high inocula to Stone Fruit

Organism	Adhesion		Attachment	
	Peaches	Plums	Peaches	Plums
Escherichia coli O157:H7	30s	0s	60s	60s
Listeria monocytogenes	60s	0s	1h	30s
Salmonella Typhimurium	0s	0s	1h	30s
Staphylococcus aureus	0s	0s	21d	1h

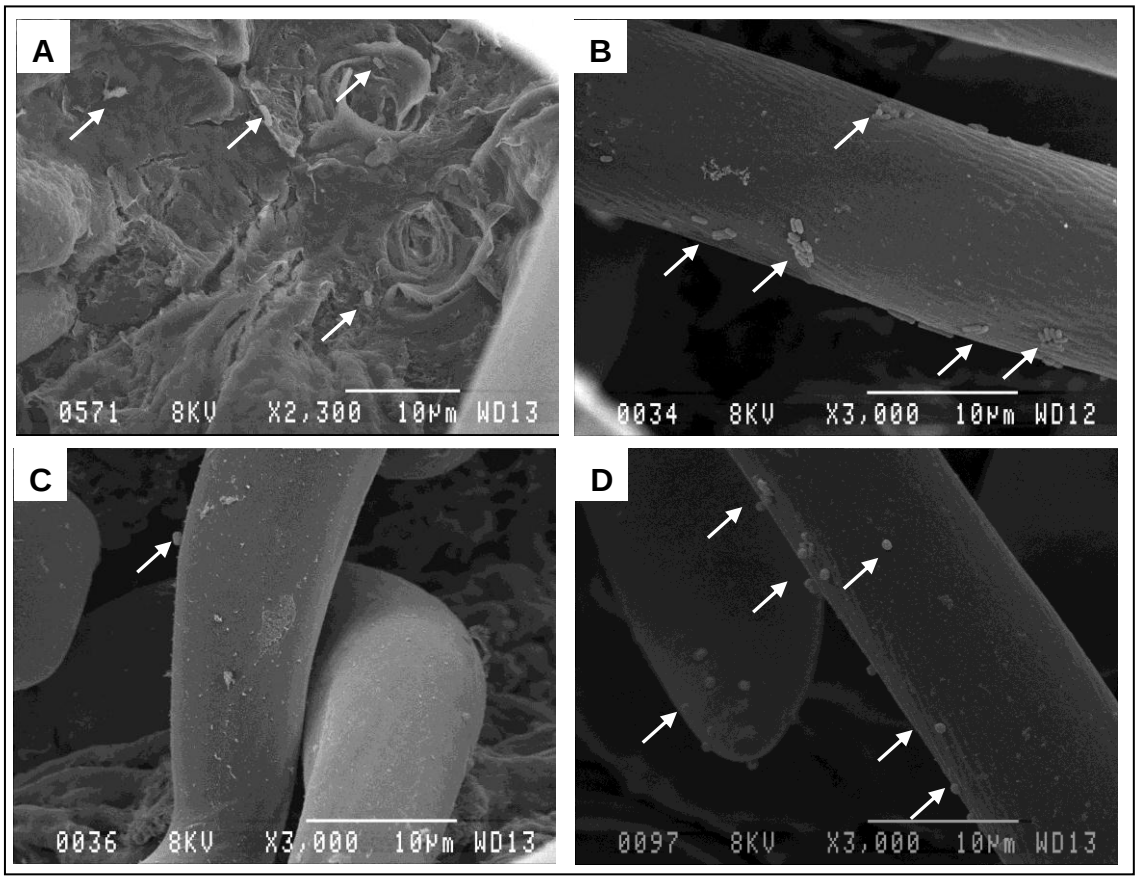

Figure 7.3: Electron Micrographs of the initial adhesion to peach surface by bacterial foodborne pathogens.

Arrows indicate the initial adhesion by *Escherichia coli* (A) to the surface of a peach. Arrows indicate the initial adhesion by *Listeria monocytogenes* (B), *Salmonella* Typhimurium (C) and *Staphylococcus aureus* (D) to the trichome of a peach.

3.3. Attachment

Attachment of bacteria was determined and defined in this study by the organism's ability to produce exopolysaccharide structures. The first attachment of *E. coli* O157:H7, due to exopolysaccharides, to peaches and plums was observed following 60s exposure (Figure 7.5A and 7.6A) (Table 7.1, Table 7.2 and Table 7.3). The first attachment of *L. monocytogenes* (Figure 7.6B) and *Salmonella* Typhimurium (Figure 7.6C) was observed on plums 30s post inoculation (Table 7.1 and Table 7.3) and to peaches, 1h after inoculation (Table 7.1 and Table 7.2) (Figure 7.5B and 7.5C). *Staphylococcus aureus* was able to visibly attach using attachment structures to the plum surface 1h post inoculation (Figure 7.6D) (Table 7.1 and Table 7.3) and to peaches, 21d following artificial contamination (Figure 7.5D) (Table 7.1 and Table 7.3). Organisms were able to attach more effectively to the plum than to the peach surface (Table 7.1, Table 7.2 and Table 7.3).

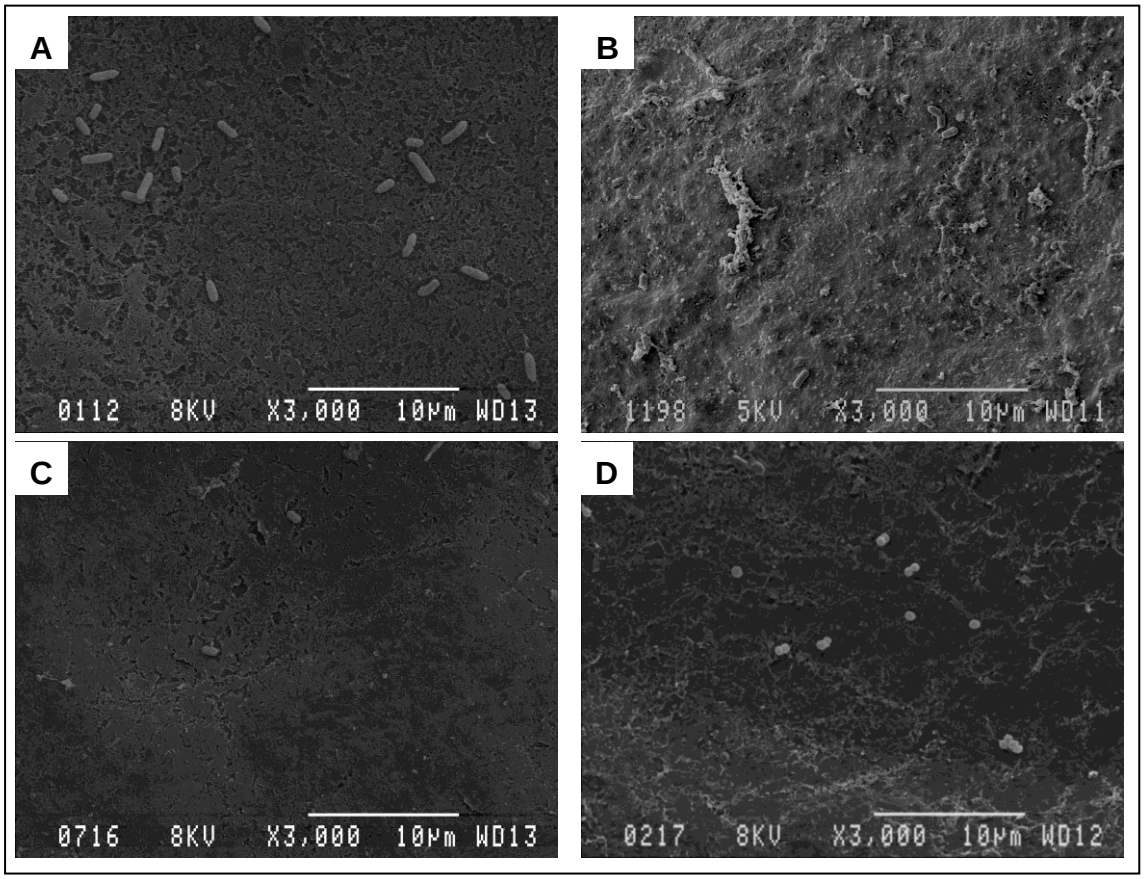

Figure 7.4: Electron Micrographs of the initial adhesion to plum surface by bacterial foodborne pathogens.

Electron micrographs illustrating the initial adhesion by *Escherichia coli* (A), *Listeria monocytogenes* (B), *Salmonella* Typhimurium (C) and *Staphylococcus aureus* (D) to the surface of a plum.

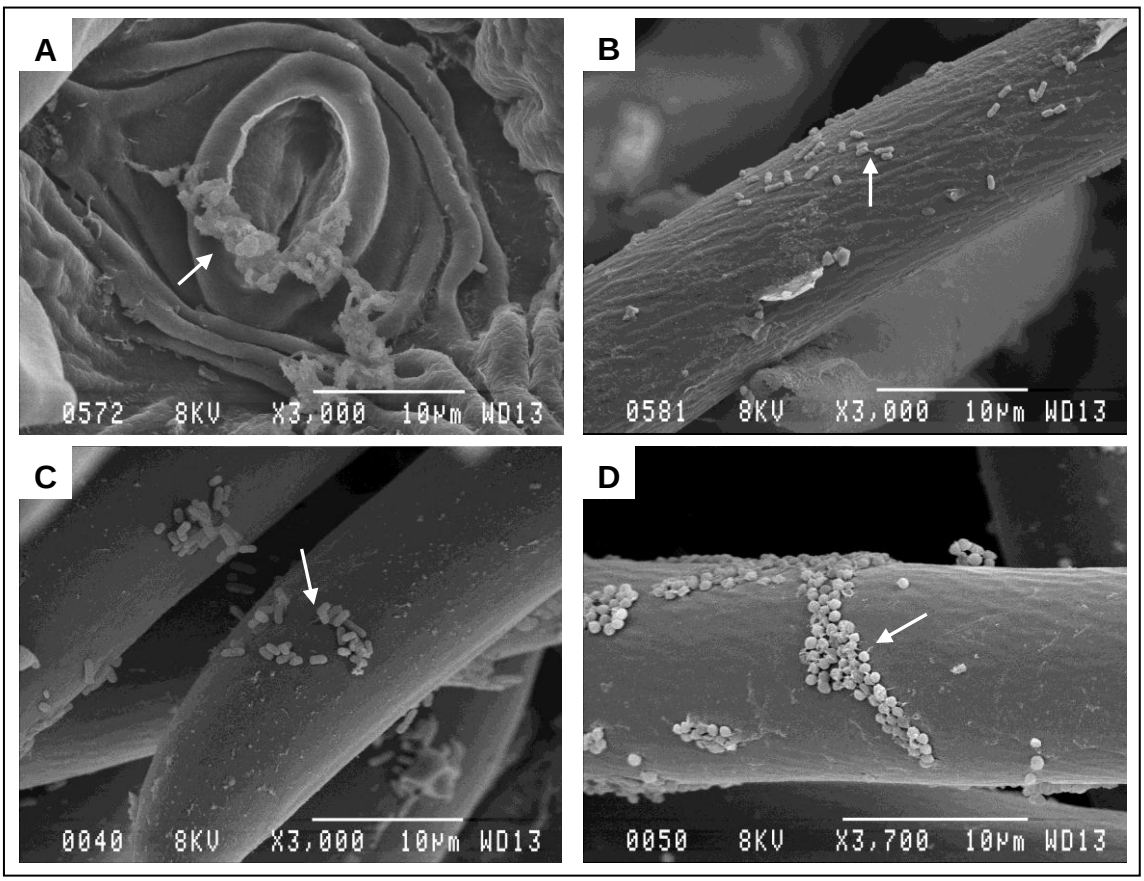

Figure 7.5: Electron Micrographs of the attachment on peach surface and surface structures by bacterial foodborne pathogens.

Arrows indicate the attachment on peach surfaces by *Escherichia coli* (A) and to peach surface structures by *Listeria monocytogenes* (B), *Salmonella* Typhimurium (C) and *Staphylococcus aureus* (D).

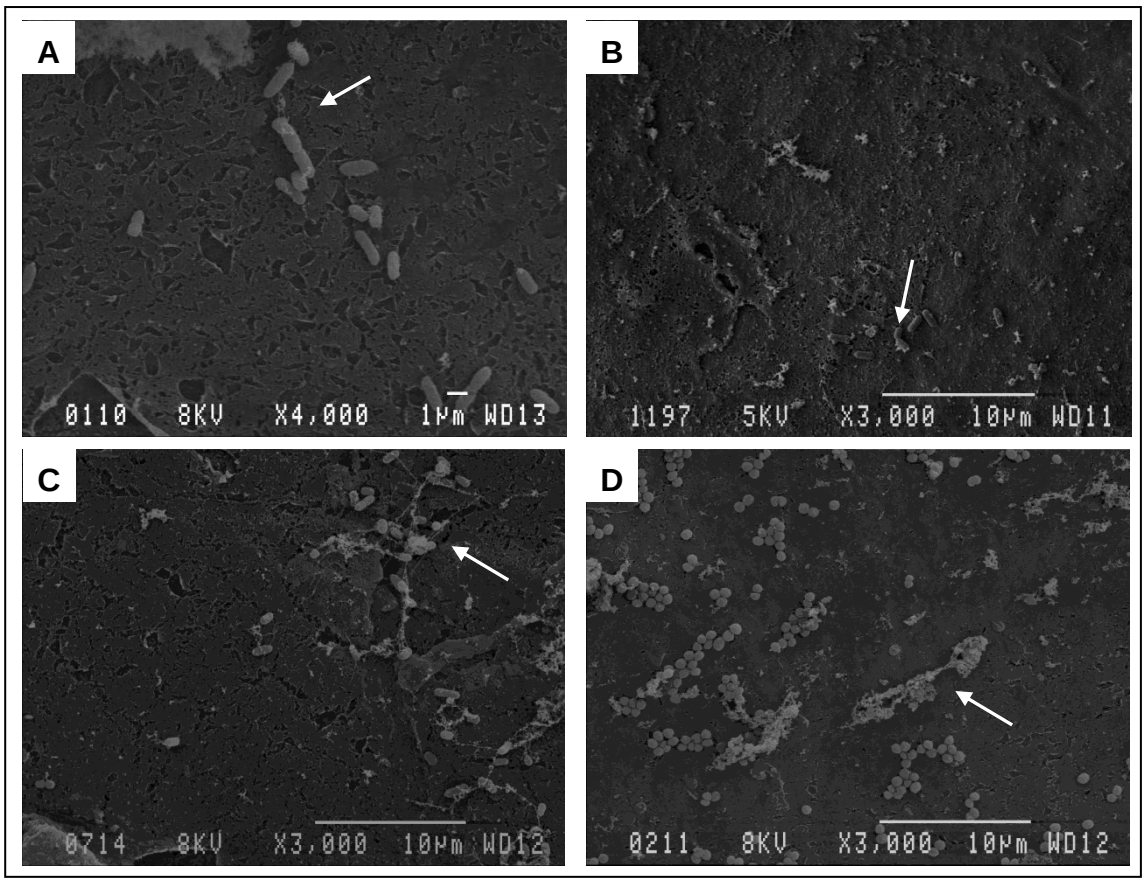

Figure 7.6: Electron Micrographs of the attachment on plum surface by bacterial foodborne pathogens.

Arrows indicate the attachment on plum surfaces by *Escherichia coli* (A), *Listeria monocytogenes* (B), *Salmonella* Typhimurium (C) and *Staphylococcus aureus* (D).

Table 7.2: Summary of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonell*a Typhimurium and *Staphylococcus aureus* attachment and replication rate on peaches following different time intervals simulating export chain conditions after exposure with high (5 log cfu/cm^2) and low (3 log cfu/cm^2) concentrations

Pathogen	Time	Attachment Rate [t]	Replication Rate [u]	SEM [v]	High [ ] [w]	Low [ ] [x]
Escherichia coli O157:H7	0s[y]	0.00%	0.00%	0.00 **d**	1.81 **bc**	*
	2h[z]	*	*	*	3.21 **a**	0.51 **ab**
	30s	0.00%	0.00%	0.49 **cd**	1.66 **bc**	*
	60s	6.67%	0.00%	1.58 **cd**	2.03 **abc**	*
	1h	6.67%	0.00%	1.47 **cd**	2.47 **ab**	*
	1d	*	*	*	2.02 **abc**	0.00 **b**
	13d	0.00%	0.00%	2.18 **bc**	0.96 **c**	0.00 **b**
	20d	6.67%	6.67%	3.99 **b**	2.23 **ab**	1.03 **a**
	21d	33.33%	6.67%	5.91 **a**	1.86 **bc**	0.07 **b**
Listeria monocyto-genes	0s[y]	0.00%	0.00%	0.00 **cd**	2.41 **bc**	*
	2h[z]	*	*	*	3.74 **a**	2.30 **a**
	30s	0.00%	0.00%	0.00 **c**	2.11 **bc**	*
	60s	0.00%	0.00%	4.10 **b**	2.49 **bc**	*
	1h	13.33%	20.00%	6.11 **b**	3.82 **a**	*
	1d	*	*	*	2.34 **bc**	0.58 **bc**
	13d	0.00%	20.00%	6.40 **a**	1.61 **c**	0.07 **c**
	20d	26.67%	33.33%	6.66 **a**	1.88 **bc**	0.81 **b**
	21d	6.67%	20.00%	6.39 **a**	3.09 **ab**	0.99 **b**
Salmonella Typhimurium	0s[y]	0.00%	0.00%	0.49 **c**	0.54 **ab**	*
	2h[z]	*	*	*	1.43 **a**	0.31 **a**
	30s	0.00%	0.00%	0.52 **c**	0.52 **ab**	*
	60s	0.00%	6.67%	4.10 **b**	0.87 **ab**	*
	1h	20.00%	13.33%	6.11 **a**	1.53 **a**	*
	1d	*	*	*	0.88 **ab**	0.00 **a**
	13d	0.00%	0.00%	6.40 **a**	0.23 **b**	0.01 **a**
	20d	0.00%	6.67%	6.71 **a**	0.75 **b**	0.04 **a**
	21d	40.00%	26.67%	6.26 **a**	0.38 **b**	0.23 **a**
Staphylococcus aureus	0s[y]	0.00%	0.00%	1.60 **c**	2.77 **cd**	*
	2h[z]	*	*	*	4.55 **a**	2.29 **a**
	30s	0.00%	0.00%	4.58 **b**	2.61 **cd**	*
	60s	0.00%	0.00%	5.61 **ab**	2.43 **d**	*
	1h	0.00%	0.00%	5.98 **ab**	3.48 **bc**	*
	1d	*	*	*	4.19 **ab**	1.74 **a**
	13d	0.00%	0.00%	6.77 **a**	4.47 **ab**	1.61 **ab**
	20d	0.00%	0.00%	1.19 **c**	3.55 **bc**	2.21 **a**
	21d	20.00%	0.00%	6.70 **a**	3.18 **cd**	0.78 **b**

[t]: percentage of observed attachment; [u]: percentage of observed multiplication [v]: LOG (counts+1) cells/cm^2; [w]: LOG (counts+1) cfu/cm^2; [x]: LOG (counts+1) cfu/cm^2 , [y]: following inoculation culture is immediately aspirated; [z]: following initial inoculation for the export chain, once dried; * value absent it was not included. Bolded small caps represent the least significant difference according to the Fischer Test (P<0.05).

Table 7.3: Summary of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonell*a Typhimurium and *Staphylococcus aureus* attachment and replication rate on plums following different time intervals simulating export chain conditions after exposure with high (5 log cfu/cm^2) and low (3 log cfu/cm^2) concentrations

Pathogen	Time	Attachment Rate [t]	Replication Rate [u]	SEM [v]	High [ ] [w]	Low [ ] [x]
Escherichia coli O157:H7	0s[y]	0.00%	6.67%	0.33 **de**	0.57 **cd**	*
	2h[z]	*	*	*	2.68 **ab**	0.74 **a**
	30s	0.00%	0.00%	0.57 **de**	0.65 **cd**	*
	60s	33.30%	33.33%	2.75 **b**	2.08 **ab**	*
	1h	46.67%	20.00%	4.22 **a**	2.90 **a**	*
	1d	0.00%	6.67%	*	2.01 **b**	0.12 **a**
	6d	*	*	0.34 **de**	0.43 **cd**	0.18 **a**
	13d	33.33%	6.67%	1.29 **cd**	0.05 **cd**	0.07 **a**
	18d	6.67%	13.33%	2.33 **bc**	0.10 **cd**	0.16 **a**
	25d	80.00%	26.67%	3.05 **b**	0.92 **c**	0.57 **a**
	27d	0.00%	0.00%	0.00 **e**	0.03 **d**	0.13 **a**
Listeria monocyto-genes	0s[y]	0.00%	0.00%	1.29 **bc**	0.71 **fg**	*
	2h[z]	*	*	*	3.04 **bc**	0.60 **b**
	30s	6.67%	0.00%	0.77 **c**	1.62 **de**	*
	60s	0.00%	0.00%	2.36 **b**	2.45 **cd**	*
	1h	13.33%	66.67%	3.93 **a**	3.62 **ab**	*
	1d	*	*	*	4.19 **a**	1.70 **a**
	6d	20.00%	33.33%	3.90 **a**	1.41 **ef**	0.13 **c**
	13d	60.00%	20.00%	4.83 **a**	1.07 **ef**	0.12 **c**
	18d	93.33%	13.33%	4.75 **a**	1.05 **ef**	0.10 **c**
	25d	53.33%	6.67%	4.15 **a**	0.09 **g**	0.02 **c**
	27d	26.67%	6.67%	1.69 **bc**	0.69 **fg**	0.03 **c**
Salmonella Typhimurium	0s[y]	0.00%	0.00%	0.94 **c**	2.08 **a**	*
	2h[z]	*	*	*	2.40 **a**	0.49 **a**
	30s	20.00%	26.67%	3.20 **ab**	1.96 **a**	*
	60s	0.00%	0.00%	2.19 **bc**	2.10 **a**	*
	1h	33.33%	40.00%	3.08 **ab**	2.36 **a**	*
	1d	*	*	*	2.81 **a**	0.22 **a**
	6d	20.00%	0.00%	2.33 **abc**	0.76 **b**	0.04 **a**
	13d	0.00%	0.00%	2.58 **ab**	0.93 **b**	0.06 **a**
	18d	40.00%	13.33%	3.70 **a**	2.08 **b**	0.00 **a**
	25d	6.67%	0.00%	2.19 **bc**	0.61 **b**	0.00 **a**
	27d	33.33%	20.00%	2.41 **ab**	0.11 **b**	0.11 **a**

Table 7.3: cont.

Pathogen	Time	Attachment Rate [a]	Replication Rate [b]	SEM [c]	High [ ] [d]	Low [ ] [e]
Staphylococcus aureus	0s[y]	0.00%	0.00%	3.50 **def**	1.85 **d**	*
	2h[z]	*	*	*	4.54 **ab**	2.51 **a**
	30s	0.00%	0.00%	2.98 **f**	2.20 **d**	*
	60s	0.00%	0.00%	3.27 **ef**	2.19 **d**	*
	1h	13.33%	0.00%	4.69 **bc**	3.64 **bc**	*
	1d	*	*	*	4.97 **a**	1.93 **ab**
	6d	100.00%	0.00%	5.67 **a**	4.44 **abc**	1.63 **bc**
	13d	60.00%	13.33%	4.89 **ab**	3.57 **c**	0.75 **d**
	18d	26.67%	0.00%	3.92 **cde**	1.94 **d**	1.23 **bcd**
	25d	26.67%	0.00%	4.27 **bcd**	2.49 **d**	1.07 **cd**
	27d	66.67%	6.67%	3.64 **def**	1.99 **d**	0.62 **d**

[t]: percentage of observed attachment; [u]: percentage of observed multiplication; [v]: LOG (counts+1) cells/cm^2; [w]: LOG (counts+1) cfu/cm^2; [x]: LOG (counts+1) cfu/cm^2; [y]: following inoculation culture is immediately aspirated; [z] following initial inoculation for the export chain, once dried; * value absent it was not included. Bolded small caps represent the least significant difference according to the Fischer Test (P<0.05).

3.4. Colonisation

For the purpose of this study, colonisation on the fruit surface was defined as the organisms' ability to reproduce on the inoculated surface area as well as the ability to form extensive attachment structures. Overtime the amount and rate of exo-polysaccharide production by *E. coli*, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* increased on stone fruit (Table 7.2 and Table 7.3). Most notable colonisation, by means of attachment structures, was observed for *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* on peach surfaces towards the end of the stone fruit export chain (Figure 7.7) (Table 7.2 and Table 7.3). Following 21d exposure, *L. monocytogenes* and *S. aureus* were able to form microcolonies (Figure 7.7B and 7.7D). No *E. coli* O157:H7 replication was observed on the peach surface between 30s and 1h (Table 7.2) and there was no significant difference in cells/cm^2 nor in cfu/cm^2 recovered from the peach fruit (Table 7.2). On plums however, *E. coli* O157:H7 replication was observed under the SEM following 60s and 1h after inoculation (Table 7.3) and a significant increase in cfu/cm^2 from 30s to 1h was evident (Table 7.3). *Listeria monocytogenes* occurred more prominently on peaches 1h post inoculation than 30s and 60s (Table 7.2) and the observed replication rate was 20% at the 1h time interval (Table 7.2). A significant increase in *L. monocytogenes* cells/cm^2 and cfu/cm^2 was observed on plums from 30s to 1h and 1d, respectively (Table 7.3) and replication was observed 1h post inoculation (Table 7.3). *Salmonella* Typhimurium was able to significantly increase on peaches and plums from 30s to 1d post inoculation (Table 7.2 and Table 7.3) with the

highest observed replication occurring 1h post inoculation on both products (Table 7.2 and Table 7.3). No significant difference was found in *S. aureus* cell counts on peaches even though a significant increase was observed in cfu/cm^2 numbers (Table 7.2). No *S. aureus* replication was visible on peaches or plums during these time intervals (Table 7.2 and Table 7.3). An overall increase in *S. aureus* cfu/cm^2 was demonstrated from 30s to 1d post inoculation on stone fruit (Table 7.2 and Table 7.3).

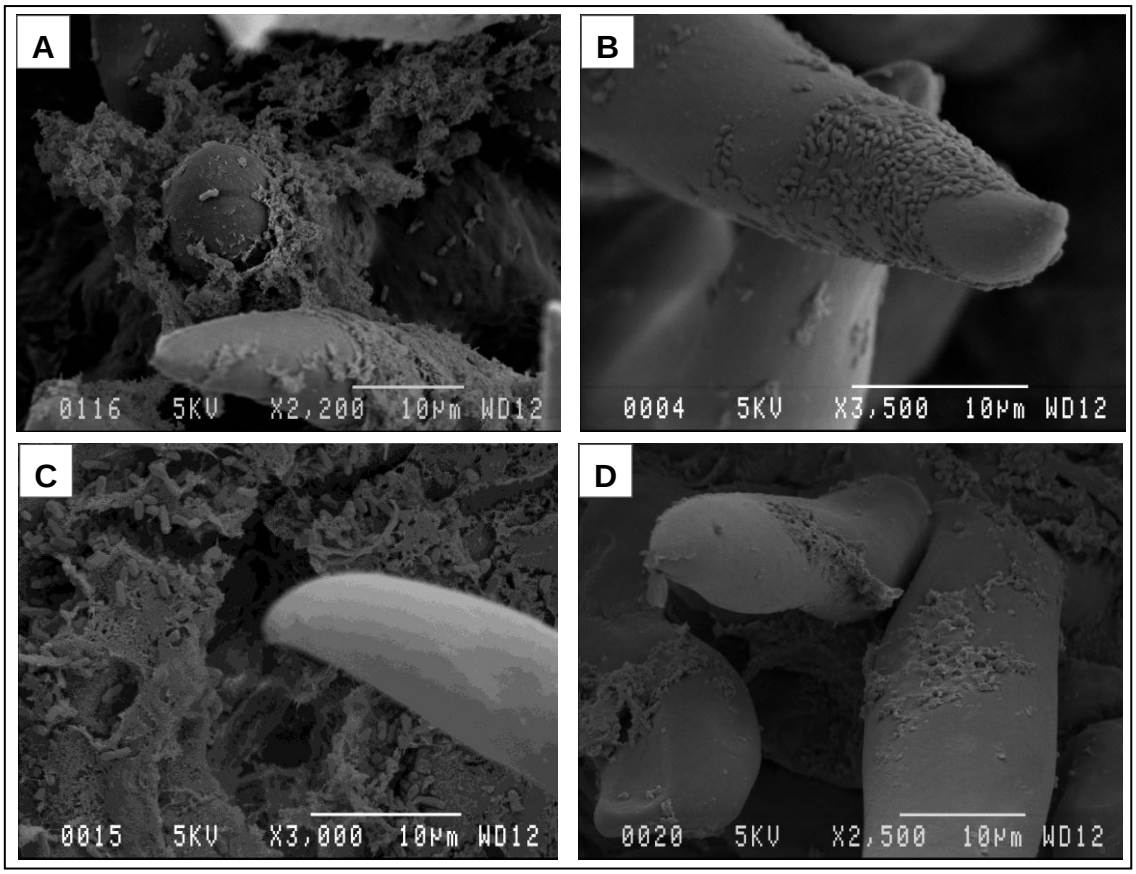

Figure 7.7: Electron Micrographs of the colonisation and survival by bacterial foodborne pathogens on peach surface.

Electron micrographs illustrating the colonisation and survival on peach surfaces by *Escherichia coli* (A), *Listeria monocytogenes* (B), *Salmonella* Typhimurium (C) and *Staphylococcus aureus* (D).

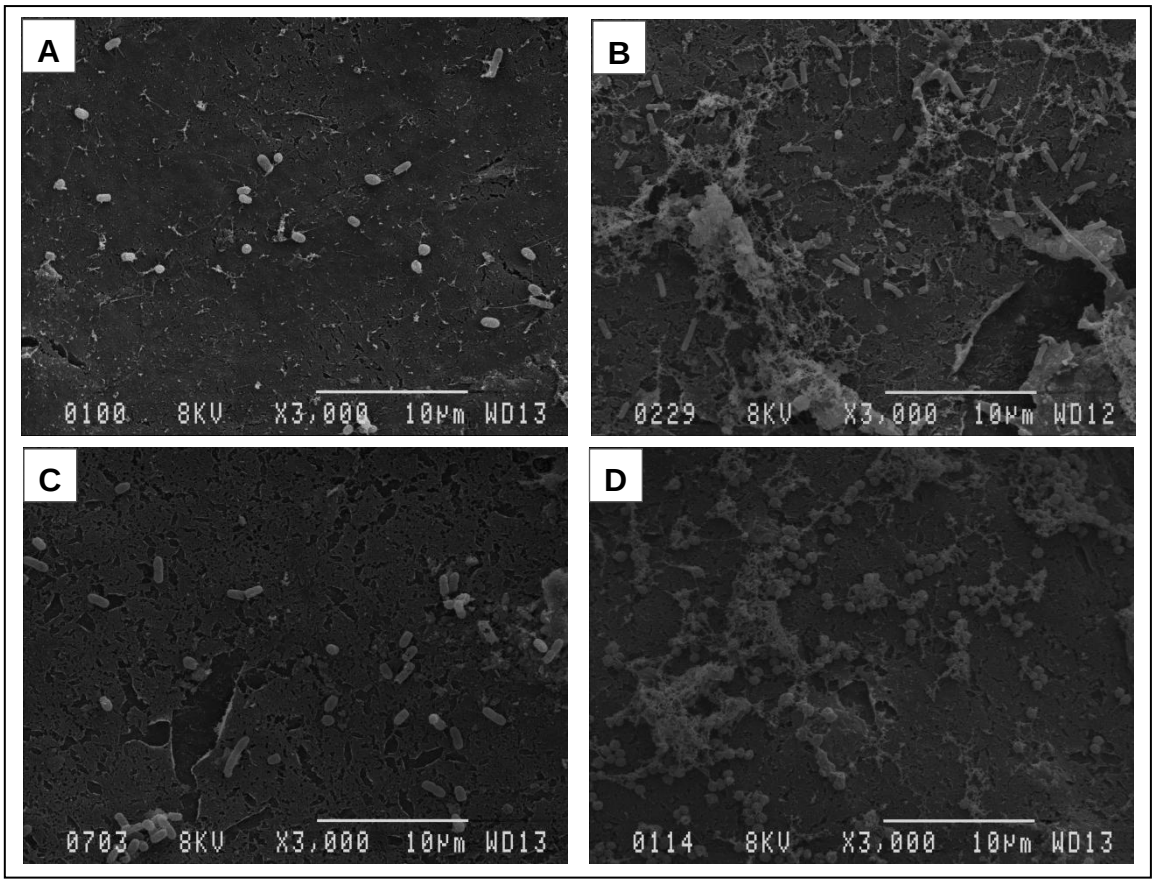

Figure 7.8: Electron Micrographs of the colonisation and survival by bacterial foodborne pathogens on plum surface.

Electron micrographs illustrating the colonisation and survival on plum surfaces by *Escherichia coli* (A), *Listeria monocytogenes* (B), *Salmonella* Typhimurium (C) and *Staphylococcus aureus* (D).

3.5. Pathogen survival

Survival was defined in this study as the organisms' ability to survive on the fructoplane throughout the simulated export chain. The 0.5^{o}C incubation conditions for a period of 1 to 13 days had no significant effect on *E. coli* O157:H7 numbers on the peach surface. However, a significant increase was observed following the 4^{o}C storage conditions for a time period of 13 to 20 days with no significant difference between 20 and 21 days (Table 7.2), even though replication was observed (Table 7.2). No significant difference was seen in *E. coli* O157:H7 titres when plums were inoculated with low concentrations (Table 7.3). However, a significant difference was observed in *E. coli* O157:H7 titre on plum surfaces when exposed to 0.5^{o}C from 1 to 6 days with the high challenge concentration inoculum (Table 7.3). Cells/cm^2 observed on the plum surface using SEM demonstrated slight but no significant increase in cell numbers, confirmed by observable consistent replication of *E. coli* O157:H7 from 30 s to 25 days (Table 7.3). *Escherichia coli* was visible under the SEM until

the completion of the simulated export supply chain (Figure 7.7A and 7.8A) A significant decrease in *L. monocytogenes* and *Salmonella* Typhimurium was observed between 1 and 6 days (0.5^{o}C) on both peaches and plums with no significant difference observed further through the simulated cold chain, indicating that an equilibrium had been where the replication and extinction rate are similar (Table 7.2 and Table 7.3). Both these organisms were also visible under the SEM until the completion of the simulated export chain (Figure 7.7B, 7.8B, 7.7C and 7.8C). *Staphylococcus aureus* titres did not reflect a significant decrease on peaches (Table 7.2), and no replication was observed under the SEM (Table 7.2). On plums there was an overall decrease in *S. aureus* numbers with the most significant decrease occurring 13d after inoculation (Table 7.3). *Staphylococcus aureus* was the foodborne bacterial pathogen which survived better than the other three foodborne bacteria and survived at higher titres through the simulated export chain (Figure 7.7D and 7.7E).

4. Discussion

This is the first study of its kind, where foodborne pathogens have attached, colonised and survived on fruit surfaces. The promotion of growth due to simulated export conditions allowed for the population under study to be sustained to levels above the minimum infectious dose. The attachment and survival of foodborne pathogens to different fresh produce surfaces has not been widely studied, but is of great significance to the food industry (Solomon and Matthews, 2006). The ability of foodborne pathogens to colonise and survive on fresh produce is dependent on their ability to adapt to ecological niches outside the host. Therefore needing to attach and colonise the niche as well as to transport and utilise available nutrients required for survival (Palumbo *et al*., 2005). Traditionally foodborne pathogens were of little importance on fresh produce, but recent reports of survival and colonisation of *E. coli* and *S. enterica* have provided evidence that contamination with these pathogens might lead to its presence and survival (Brandl, 2006). Fett (2000) suggested that human pathogens may become incorporated into phylloplane biofilms allowing the organisms to buffer environmental fluctuations (Marshall, 1992; Monier and Lindow, 2005; Morris and Monier, 2003). These biofilms are often associated with sources of nutrients such as leaf vein and trichomes (Monier and Lindow, 2005). Plant surfaces and bacterial cell are both negatively charged (Van Loosdrecht *et al.,* 1990), therefore a natural repulsive force exists. Adhesion occurs when bacterial cells are able to overcome these natural repulsive forces (Garret *et al.*, 2008; Van Loosdrecht *et al.,* 1990). Adhered cells then become attached by means of exopolysaccharides. Once attached, cells are able to replicate to form microcolonies (Lindsay and Holy, 2006). Microcolonies may lead to survival. Most studies to

date focus on processed fruit in the post-harvest environment and not on pre-harvest contamination or on crops within the supply chain (Brandl, 2006).

In this study it was found that all pathogens adhered to stone fruit surfaces within 60 seconds. Solomon and Matthews (2006) demonstrated that heat-killed bacteria could adhere to lettuce leaves, demonstrating that no physiological activity is required for adhesion. In general, adhesion of *E. coli* O157:H7 and *L. monocytogenes* occurred more rapidly to plum surfaces than to peach surfaces. Attachment structures were seen at earlier time intervals on plums than on peaches. In this study, attachment occurred as early as one minute for *E. coli* O157:H7. *Listeria monocytogenes and Salmonella* Typhimurium had a better ability to attach and grow on plums than *E. coli* O157:H7, which is in agreement with Barak *et al.* (2002). The same trend however was not seen with peaches, where *E. coli* O157:H7 had a better ability to attach and grow on than *Salmonella* Typhimurium. Barak *et al.* (2007) and Jeter and Matthysee (2005) demonstrated that *E. coli* O157:H7 and *Salmonella* spp. were able to produce fibrils and aggregative polymers for attachment. Plant pathogens produce similar fibrils to attach to plant hosts, Latham *et al.* (1978) demonstrated that *Pseudomonas lachrymans* was demonstrated to attach to young cucumber leaves after 10 minutes and *Ruminococcus flavefaciens* were able to attach to ryegrass following 30 minutes exposure (Leben and Whitmoyer, 1979). Similar lengthy attachment times (two hour) were reported when *Bacillus subtilis* was studied on avocado leaf surfaces (Demoz and Korsten, 2006). Differences in attachment can be attributed to different inoculation and quantification methodology as well as the initial concentration of the organism on the surface of the test commodity, differences in the fructo- and phyllo-plane and the differences in pathogen characteristics. Adhesion and attachment are essential prior to colonisation and survival. Attachment is considered a mechanism to ensure that the bacterial cells are not dislodged from the surface once the colonisation phase is triggered. Following the initial interaction (adhesion) between the bacteria and the plant, attachment follows if the organism is able to utilise the surface nutrients.

Bacteria in the present study were able to attach to one another, forming typical microcolonies. Barak *et al.* (2002) also demonstrated that *S. enterica* not only used colonisation niches on sprout surfaces but also showed patterns of attachment to one another, therefore increasing the possible attachment surface area.

Foodborne pathogens used in this study were found to colonise peach surfaces more effectively than the plum surface. Trichomes on the peach surface serve as additional colonisation sites for microorganisms thereby increasing the surface area that could be used

for adherence, attachment and eventual colonisation. The presence of trichomes also provided for increased bacterial niche protection making detachment more difficult during washing. The colonisation studies using the four selected foodborne pathogens indicated preferential sites on the peach trichomes and nearby lenticels. Seo and Frank (1999) demonstrated that *E. coli* O157:H7 and epiphytes that attached to the intact surface of lettuce leaves attached to areas located near stomata's, on trichomes and on veins. Takeuchi and Frank (2000) demonstrated that plant pathogens may be better adapted to the phyllosphere than human foodborne bacterial pathogens. In this study, *Salmonella* Typhimurium and *E. coli* O157:H7 were the least effective colonisers of stone fruit surfaces.

In the current study it was demonstrated that *Salmonella* Typhimurium was able to produce microcolonies but the survival of the organism was poor. *Salmonella enterica* was observed to form colonies on cilantro leaves two days post inoculation, with larger colonies nine days post-inoculation (Brandl and Mandrell, 2002). *Salmonella* Typhimurium produced microcolonies to a lesser degree than *S. aureus* and *L. monocytogenes* on the observed sections. Microcolony formation is one of the survival strategies used by bacteria cells, to provide protection rendered by the exopolysaccharides (Leigh and Coplin, 1992).

Another important aspect required for effective microbial colonisation is the ability to multiply on the surface of the fruit, once attached. In this study, multiplication of all four foodborne bacterial pathogens was observed on the stone fruit surface areas, demonstrating the bacterial ability to utilise available nutrients on the surface of the fruit. The increase in numbers of *S. aureus* also demonstrates the organism's ability to reproduce on the peach fruit surfaces. Colonisation and survival of enteric bacteria was demonstrated on plants by various authors (Brandl and Mandrell, 2002; Islam *et al.*, 2004a; Islam *et al.*, 2004b; Natvig *et al.*, 2002; Solomon *et al.*, 2003), but mainly on leaves and roots.

Following the initial export temperature of 0.5^{o}C, all pathogen's titres tested in this study, except *S. aureus,* decreased over time on peaches and plums. The drop in viable counts could be directly linked to the lowered temperature. Survival of foodborne pathogens can therefore be reduced by careful managing and maintaining correct export temperatures of stone fruit at 0.5^{o}C. However, pathogen titres in this study were found to increase again once the fruit was removed from cold storage conditions, simulating the export chain. Similarly, Francis and O'Beirne (2001) found a decrease in titre of *E. coli* O157:H7 and *L. monocytogenes* when comparing growth of the organisms when changing from 8^{o}C to 4^{o}C. At high conducive temperatures (optimum temperatures) and relative humidity *S. enterica* was able to multiply rapidly on the phyllosphere (Brandl and Mandrell, 2002). It was

determined from this study that *E. coli* O157:H7 inoculated onto stone fruit at realistic contamination loads will not survive the entire export chain, when contaminated at the point of harvest, if the correct cold chain regimes are adhered to. Survival of *E. coli* O157:H7 in this study was poor, even though the organism was able to adhere, attach and colonise. Mitra *et al.* (2009) found that *E. coli* spot inoculated on spinach leaves followed the same trend but Solomon *et al.* (2003) demonstrated that the *E. coli* O157:H7 population declined, but the organism was able to survive on lettuce seedlings for up to 30 days post inoculation. Temperatures also influenced *E. coli* O157:H7's survival in this study with titres decreasing following the ultra-low temperatures (0^{o}C) with slight recovery following refrigeration temperatures (at high concentrations).

Listeria monocytogenes and *S. aureus* survived on the stone fruit surfaces more effectively than the other two pathogens studied. *Listeria monocytogenes* is able to survive freezing temperatures and *S. aureus* is an organism that can withstand a number of environmental stresses in its natural habitat (human skin). A fluctuation in bacterial numbers was observed over the period immediately after inoculation at 21^{o}C with a decrease in titre when placed at 12^{o}C (peaches) or 7.5^{o}C (plums) storage. *Listeria monocytogenes* was found to down regulate attachment ability at 37^{o}C and had optimal colonisation and survival at temperature of 20^{o}C followed by 30^{o}C and then 10^{o}C (Gorski *et al.*, 2003).

In this study, *S. aureus* was unable to survive at high enough numbers known to produce toxins. However, when inoculated with unnaturally high inoculum dosages the pathogen was able to survive at high enough concentrations that may enable the organism to potentially produce toxins. The likelihood of this scenario ever happening under natural circumstances is unlikely but it shows the potential for the organism to be able to maintain initial titres. If high enough levels can be maintained it has the potential to produce toxins. Following the simulated export chain, *E. coli* O157:H7 was able to survive at titres that could potentially lead to foodborne illness since the minimal infectious dose of *E. coli* O157:H7 is 10^{1} cells. *Listeria monocytogenes* was seen to survive at 10^{3} cells which has been described as a level high enough to possibly cause illnesses (FDA, 2009). *Staphylococcus aureus* requires presence of 10^{5} cells in order to produce toxins (FDA, 2009). Future research will focus on the likelihood of potentially causing consumer illnesses at the end of the supply chain.

In conclusion, in order for illness to result from the consumption of contaminated fresh produce, foodborne pathogens needs to adhere, attach, colonise and proliferate to a high enough concentration above the minimum infectious dose. If fresh produce is contaminated

preharvestly, the organism needs to survive through postharvest treatments including export cold chain storage conditions. If the organism is able to survive on the fruit surface under export environmental conditions and then proliferate prior to consumption, the level of risk increases. *Salmonella* Typhimurium and *S. aureus* in pure culture inoculated onto fruit under laboratory conditions represent a low risk scenario of foodborne pathogen contamination on stone fruit. Evidence from this study demonstrates that *E. coli* O157:H7 and *L. monocytogenes* are able to survive and retain high enough levels on stone fruit surfaces under controlled conditions that are above the minimum infectious dose. However, this scenario can only happen when fruit is artificially contaminated with an unnatural high inoculum load that makes detection and monitoring possible. The authors doubt that this scenario will imitate natural infection models and lower inoculum loads are more likely prevail in nature. But, this directly shows the potential of cross infection, colonisation and survival potential. The time-temperature regime for high concentrations of *E. coli* O157:H7 and *L. monocytogenes* on stone fruit would therefore require preventative intervention strategies. However it is unrealistic to conclude that such artificially high concentrations will ever occur under basic good agricultural practices.

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Chapter 8

Microbial risk profiles of the South African peach and plum export industry

Stacey Collignon and Lise Korsten

Abstract

Microbiological risk profiles of plums and peaches exported to the United Kingdom and the European Union were assessed using the Risk Ranger. *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* were identified as potential hazards of interest in peach and plum production. A hazard characterisation, exposure assessment and risk characterisation were done based on available literature. A semi-quantitative assessment was conducted using the Excel based assessment tool, Risk Ranger. A risk profile of the identified hazards, pairing with peaches and plums was created and expressed as risk to the final consumer. This semi-quantitative tool allowed an unbiased and unambiguous rating of risk and a comparison of consequences that the effectiveness of the post-processing control system has on the risk to the final consumer. Peaches possibly contaminated with *L. monocytogenes* were considered a potential high risk to the extremely susceptible consumers in the scenario of uncontrolled post-processing control system export to the UK. Peaches potentially contaminated with *L. monocytogenes* in a well-controlled export safety system to the United Kingdom pose only a medium risk to both population groups (general and extremely susceptible). Peaches possibly contaminated with *L. monocytogenes* exported to the European Union with a controlled or un- controlled post-processing control system pose a medium risk to consumers. Peaches exported to the United Kingdom and potentially contaminated with *E. coli* O157:H7, *Salmonella* Typhimurium or *S. aureus* were considered to only pose a low or medium risk to consumers. Plums exported to the United Kingdom and the European Union and potentially contaminated with *Salmonella* Typhimurium or *S. aureus* pose a low risk to the consumers. Whereas, plums exported to the United Kingdom or European Union and potentially contaminated with *E. coli* O157:H7 or *L. monocytogenes* pose either a low or medium risk. These results can comprise a source of information to be used for the improvement and proper implementation of appropriate food safety management practices. It also allows the peach and plum export industry to identify critical gaps and focus research on quantitative risks involved.

1. Introduction

Fresh produce (fruit and vegetables) is an important export commodity of South Africa with 1882.47 million dollars earned in the 2007 season (FAO, 2010). A high volume of peaches and plums are produced in South Africa which enter the supply chain into three possible avenues; export sale, local sale and processing. Export sale is the largest for plums with an average of 43 494 tonnes exported from 2005 to 2009, followed by local sales at 20 777 tonnes (FAO, 2011). Approximately 54.87% of plums exported are sent to the UK, with 30.5% to the EU (PPECB, 2010). Peaches have the highest volume entering the domestic market with an average of 47 599 tonnes sold locally, followed by 7 888 tonnes traded on international markets (FAO, 2011). Approximately 33.63% of internationally sold peaches are exported to the UK and 22% to the EU (PPECB, 2010). These large volumes of international and domestic sales and consumption of peaches and plums require adequate food safety assurance for consumers.

Foodborne outbreaks are increasingly being associated with fresh fruit and vegetables (Todd, 1997), but at the present time and to the authors' knowledge peaches and plums have never been implicated as the source of an outbreak. *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. have been associated with disease outbreaks after the consumption of contaminated cantaloupe, cut fruit, strawberries, tomatoes, spinach, and various other fresh produce (Brandl and Mandrell, 2002; CDC, 2005; CDC, 2006a; CDC, 2006b; CDC, 2007; CDC, 2008; Herwaldt *et al.*, 1994; Korsager *et al*., 2005; Le Guyader *et al.*, 2004; Seymour and Appelton, 2001].

Escherichia coli O157:H7, *L. monocytogenes* and *Staphylococcus aureus* have been recorded to be present on stone fruit tested from SA (refer to Chapter 4 and Chapter 5) and *E. coli* and *Salmonella* spp. have previously been detected on the surface of stone fruit after the random sampling of fruit (Abdelnoor *et al.*, 1983). These foodborne pathogens could have been present on the surface of the stone fruit due to a number of possible contamination factors, including contaminated water, hands or surfaces (Brackett, 1999; James, 2006; Rajkowski and Baldwin, 2003). *Escherichia coli* O157:H7, *L. monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *S. aureus* have been shown to adhere, attach and colonise the peach and plum surfaces but none of the pathogens were able to proliferate under strictly controlled and simulated export or domestic supply chain conditions (Collignon and Korsten, 2010 and Chapter 6). Even though these organisms do not proliferate only a decrease in titres was recorded and therefore a complete elimination of the hazard does not take place. Therefore contamination should be prevented by

implementing various food safety management systems within the agricultural sector. A number of systems, like good agricultural practices, Hazard Analysis and Critical Control Points and other quality assurance standards, systems and guidelines, have the potential to allow for the prevention of contamination. It also prevents further pathogenic proliferation and survival through the supply chain if managed and controlled correctly and effectively. In the event that these systems and standards fail it is possible that consumers could be at risk. An aim of this study was to determine if a well-controlled post-processing control system poses less risk to the final consumer than a system that is not controlled.

Due to the large volume of peaches and plums exported from South Africa, a risk profile was conducted to provide risk ratings of hazard-peach and hazard-plum pairings. A risk profile using the Risk Ranger (Ross and Sumner, 2002) was conducted due to the lack of quantitative data available to conduct a full quantitative risk assessment and as a prelude to recommendations to the industry as to where to focus future research efforts. This is the first report where the Risk Ranger, available freely from the United Nations Food and Agricultural Organisation, has been used to determine the risk that hazards play in peach and plum consumption. This study it was important to determine the level of risk to final consumers in export markets within the UK and EU, under various population groups.

2. Methodology

2.1. Hazard Identification

The risk assessment process began by identifying which microbiological hazards to focus on. Thus identifying which microorganisms could possibly be present as hazards on stone fruit. In this study it was decided to focus on four particular hazards, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* as important organisms in the potential contamination of fresh produce [Brandl and Mandrell, 2002; CDC, 2008; CDC, 2007, CDC, 2006a; CDC, 2006b; CDC, 2005; Herwaldt *et al.*, 1994; Korsager *et al*., 2005; Le Guyader *et al.*, 2004 and Seymour and Appelton, 2001]. *Staphylococcus aureus* was selected as an organism to indicate poor personal hygiene (Aarnisalo *et al.,* 2006).

2.2. Exposure Assessment

2.2.1. Pathogen prevalence and concentration

In this study, the hazard identification and pathogen prevalence on fruit and within the growing environment was assessed (as outlined in Chapter 4 and Chapter 5), as there is no

previous data exploring the presence of these pathogens on peaches or plums or within the growing environment. The overall prevalence of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* on the two peach farms in this study was 4.63%, 0.69%, 0% and 1.85% from all samples tested from the two peach farms, respectively but only *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* were detected on 1% of peach fruit samples tested (as outlined in Chapter 4 and Chapter 5). Plum farms, in this model, had an overall prevalence of 5.65%, 0.68%, 0% and 1.22% of all samples tested, respectively with only *L. monocytogenes* detected on 2% of plum fruit samples (as outlined in Chapter 4 and Chapter 5). The concentration upon detection of the hazard on the product, in this risk profile, was assumed to be at the lowest detectable limit tested on peaches and plums, which was log 2 or 100 cells per fruit (as outlined in Chapter 3).

The infectious dose for *E. coli* O157:H7 has previously been recorded as 1 to 100 cfu (Paton and Paton, 1998). Therefore, for the general population the infectious dose would be considered 1 000 cfu and for the extremely susceptible it would be considered 10 cfu. According to the FDA (2009a) the infectious dose of *L. monocytogenes* is dependent on the bacterial strain and susceptibility of the victim. For susceptible persons the infectious dose is considered fewer than 1 000 cfu. In this study it was considered that the infectious dose for the general population was 10 000 cfu and for the extremely susceptible it was 1 000 cfu. The infectious dose of *Salmonella* spp. is difficult to determine and for the purposes of this study the infectious dose decided on for the general population was 100 cfu and for the extremely susceptible it was 15 cfu (FDA, 2009b). The toxin that causes staphylococcal food poisoning is only produced at a high enough concentration when the *S. aureus* population exceeds 100 000 (FDA, 2009c). When the supply chain had “no effect” on the hazard, then the concentration of the hazard after the supply chain was considered 100 cfu/fruit (refer to Chapter 3, Chapter 6 and Chapter 7). When the supply chain caused a “slight reduction” then it was assumed that the concentration after the supply chain was 10 cfu/fruit and when the supply chain “usually eliminated” the hazard then it was assumed to be 1 cfu/fruit (refer to Chapter 3, Chapter 6 and Chapter 7). The required infectious doses and the “increase required to cause illness” was calculated accordingly.

2.2.2. Food consumption patterns

Food consumption estimations relied on FAOSTAT statistical data as an average over one year, although consumption of peaches and plums are focused in approximately four months during the growing season. The average export of peaches from 2005 to 2009 was 7 888 tonnes per year. European regulations (EU 543/2011) states that peaches with a minimum

diameter of 51mm (65g) and a maximum diameter of 90mm (300g) and plums with a diameter from 35 to 70mm (40 to 180g) can be sold into the EU (including the UK) (Commission Implementing Regulation EU 543/2011, 2011). 2 652.73 tonnes (33.63% of 7 888 tonnes) of peaches are exported to the UK if exported peaches from South Africa have an average weight of 182.5g then an estimated 14 575.44 peaches are exported into the UK. The EU imports an estimated 1 735.36 tonnes (22% of 7 888 tonnes) of South African peaches per year, which equates to 9 640.89 peaches (if an average exported peach weighs 182.5g). The average export of plums from 2005 to 2009 was 43 494 tonnes of plums of which 54.87% were exported to EU (n=9 568.68 tonnes which equates to 86 988 plums, if one plum weighs 110g) and 30.5% were exported to the UK (n=13 265.67 tonnes which equates to 120 597 plums). The population of the UK is 62 300 000 at the last estimate in July 2010 (Office of National Statistics, 2011) and the population of the EU is 425 418 981 at the last estimate in January 2011 (Eurostat, 2011). The number of grams fruit consumed per year was calculated based on 5%, 25%, 75% and 100% of the population according to the selections of Risk Ranger. The best percentage of the population consuming between one and five fruit per year was selected. It was determined that 5% of the EU population will consume one SA grown peach per year and 25% of the population will consume three SA grown plums in one year. In the UK, 25% of the population will consume one SA grown peaches a year and 75% of the UK population will consume 4 plums per year (Table 8.1).

Table 8.1: Summary of calculations for the consumption of peaches and plums in the European Union and the United Kingdom

Description	**Unit**	**Export to EU**		**Export to UK**	
		Peach	**Plum**	**Peach**	**Plum**
Average from 1988 to 1998	tonnes	1735.36	23865.16	2652.734	13265.67
Population	people	425418981		62300000	
Total consumption per year	g	81.584	1121.960	227.093	425.864
Fruit consumed per year per person	fruit	1	3	1	4
Portion of the population	%	5%	25%	25%	75%

Extremely high risk populations like those living with HIV make up 0.14% of the UK's population (n=114 766 people living with HIV) (AVERT, 2010a) and 0.5% of the EU's population (n=2 300 000 people living with HIV) (AVERT, 2010b).

2.3. Risk Characterisation

Hazard severity (question 1) was assessed based on available literature (Mataragas *et al.*, 2008; Sumner *et al.*, 2005). *Escherichia coli* O157:H7 was classed as a moderate hazard for both the general and the extremely susceptible population. *Listeria monocytogenes* was classified as a moderate hazard for all population types. *Salmonella* spp. was classified as a mild hazard for all population groups and *S. aureus* was classified as a minor hazard for all population groups (Mataragas *et al.*, 2008; Sumner *et al.*, 2005). The probability of contamination of the raw product per serving (question 6) and the increase needed in post-processing contamination level to cause infection or intoxication (question 7) was based on the pathogens prevalence and concentration data.

The susceptibility of the population (question 2) was compared between the general population and the extremely susceptible groups (HIV and AIDS). The percentage of the population that is extremely susceptible is discussed in section 2.2 under food consumption patterns. Frequency of consumption (question 3), proportion of consuming population (question 4) and size of the consuming population (question 5) relied on section 2.2's food consumption patterns.

The potential for post-processing contamination (question 8) and how effective is the post-processing control system (question 9) was based on the opinion and experience of the authors. Food preparation (question 11) plays no role in fresh produce consumption and contamination as these fruit types are consumed without cooking or other preparations but often fruit are washed prior to eating. Therefore the food preparation in this regard was the effect of washing. The estimated effect of home washing of fruit was a 1 log decrease in the microbial load, as was illustrated by Kilonzo-Nthenge *et al.* (2006) who determined a 0.88 log on apples and tomatoes artificially inoculated with *L. innocua* (a surrogate for *L. monocytogenes*), following a rinse and rub under cold running water.

Peaches and plums undergo no processing when being sold as a whole fruit therefore the effect that the supply chain has on the hazard potentially contaminating the fruit after the local or export supply chain was considered for the post-processing effect on the hazard (question 7). The effect of the local and export supply chain on the hazard can be obtained in Chapter 6 and Chapter 7. Question 10 (the increase required to cause infection) was based on the pathogen prevalence and concentration (section 2.2) and the effect of the local or export chain on the hazard.

Risk was characterised as low when the Risk Ranger ranking was less than 32, medium when the Risk Ranger ranking was between 32 and 48, high when the Risk Ranger ranking was between 48 and 60 and very high if the Risk Ranger ranking exceeded 60.

3. Results

Table 8.2 and Table 8.3 present the risk ratings and estimated potential illnesses following the consumption of peaches and plums possibly contaminated with *E. coli* O157:H7 in the international market (UK and EU).

South African grown peaches possibly contaminated with *E. coli* O157:H7 pose only a medium risk to general and extremely susceptible consumers of the UK irrespective of the post-processing control system (Risk ranking well controlled: 33 and 39 and not controlled: 35 and 41). The risk to consumers consuming peaches that are potentially contaminated with *E. coli* O157:H7 under a well-controlled post-processing control system is low (Risk ranking general population: 29 and extremely susceptible: 31) but if the post-processing system is not well controlled the risk increases to a medium risk (Risk ranking general population: 35 and extremely susceptible: 37). Due to the small number of people consuming SA grown peaches in the UK (only 25% a few times per year) and the EU (only 5% a few times per year), the number of illnesses that might result due to consumption of SA grown peaches is 4.67 for the general population and 0.017 for the extremely susceptible in the UK or 6.38 for the general population and 0.069 for the extremely susceptible in the EU (Table 8.2). With a post-processing control system that might not be working efficiently, there is a ten-fold increase in number of illnesses predicted due to the *E. coli* O157:H7-peach pairing (Table 8.2).

A greater percentage of the population of the UK and EU consume plums when compared to peaches (Table 8.2 and 8.3). The hazard-product pairing of *E. coli* O157:H7-plums only poses a low or medium risk to the general population and extremely susceptible populations in the UK (Risk ranking: 30 and 32) and the EU (Risk ranking: 28 and 29) when a well control post-processing control system is in place. In addition, a medium risk exists when the post processing control system is not well controlled, in the UK (Risk ranking: 36 and 38) and the EU (Risk ranking: 33 and 35) consumer models (Table 8.3). Fewer illnesses were predicted to occur following the consumption of plums potentially contaminated with *E. coli* O157:H7 than following the consumption of contaminated peaches. According to estimates obtained when using the assessment tool, only 1.4 illnesses would result in the general population and 0.005 in the extremely susceptible population of the UK and only 3.19 in the general

population and 0.0034 in the extremely susceptible population of the EU when a well-controlled post-processing control system is in place (Table 8.2). As with peaches, the number of illnesses predicted increased ten-fold when the post-processing control system was not managed effectively (Table 8.2).

In Table 8.4 and Table 8.5 are presented the risk ratings and estimated illnesses for the *L. monocytogenes*-product pairings within the UK and EU after the consumption of peaches and plums produced in South Africa.

In the UK only 25% of the population will consume a SA grown peach a few times per year. The risk to the general and extremely susceptible UK population following consumption of a potentially contaminated *L. monocytogenes* peach is medium (Risk ranking: 37 and 45) when post-processing control system is properly implemented and managed. If the post-processing control system is not functioning correctly then the risk to the general UK population (Risk ranking: 43) remains medium but to the extremely susceptible UK population (Risk ranking: 51) is high. In the general population an estimate 23.4 illnesses will result due to *L. monocytogenes* contamination of peaches and 0.86 illnesses in the extremely susceptible portion of the population with a controlled post-processing control system (Table 8.4). A tenfold increase in number of predicted illnesses was determined when the post-processing control system is not functioning efficiently (Table 8.4). Of the EU population only 5% will consume a South African grown peach a few times per year, this analysis resulted in a medium risk to the general population (Risk ranking: 33 or 39) and to the extremely susceptible population (Risk ranking: 41 or 47) irrespective of the post-processing control system (Table 8.4). In the EU it was predicted that only 23.4 illnesses may result after the consumption of *L. monocytogenes* contaminated plums from SA and only 0.86 of the extremely susceptible population were predicted to fall ill (Table 8.4). Again a ten-fold increase in the number of predicted illnesses will result when the post-processing control system is not adequately controlled (Table 8.4).

Table 8.2: Risk ranking summary of *Escherichia coli* O157:H7 contamination of South African grown peaches locally and following export to the United Kingdom and the European Union for the low and high susceptible consumers, taking the effectiveness of the post-processing control system into account

Risk Ranger Question[a]	United Kingdom Risk				European Risk			
	Controlled Post-processing Control System		Poorly Controlled Post-processing Control System		Controlled Post-processing Control System		Poorly Controlled Post-processing Control System	
	Low	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
Q1	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Q2	General	Extremely	General	Extremely	General	Extremely	General	Extremely
Q3	Few	Few	Few	Few	Few	Few	Few	Few
Q4	25%	25%	25%	25%	5%	5%	5%	5%
Q5	62300000	114 766	62300000	114 766	425418981	2300000	425418981	2300000
Q6	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent
Q7	Usually eliminates	Usually eliminates	Usually eliminates	Usually eliminates	Usually eliminates	Usually eliminates	Usually eliminates	Usually eliminates
Q8	No	No	No	No	No	No	No	No
Q9	Well controlled	Well controlled	Not controlled	Not controlled	Well controlled	Well controlled	Not controlled	Not controlled
Q10	100x	10x	100x	10x	100x	10x	100x	10x
Q11	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction
Probability of illness[c]	8.22E-10	1.64E-06	8.22E-09	1.64E-05	8.22E-10	1.64E-06	8.22E-09	1.64E-05
Total predicted illnesses[d]	4.67E+00	1.72E-02	4.67E+01	1.72E-01	6.38E+00	6.90E-02	6.90E-02	6.9E-01
Risk ranking value	33	35	39	41	29	31	35	37
Risk Ranking[e]	**Medium**	**Medium**	**Medium**	**Medium**	**Low**	**Low**	**Medium**	**Medium**

[a]:Q1, hazard severity; Q2, susceptibility of population; Q3, product consumption frequency; Q4, proportion of population consuming that product; Q5, size of consuming population; Q6, probability of contamination of raw product; Q7, effect of processing on the hazard, Q8, potential for recontamination of product after processing; Q9, effectiveness of post-processing control system; Q10, increase of hazard needed to cause illness; Q11, preparation of product before eating. [b]: High risk and low risk population groups, [c]: per day per consumer of interest, [d]: Per annum in population of interest, [e]: Risk <32 is considered **low**, risk between 32 and 48 is considered **medium**, risk between 48 and 60 is considered **high** and risk >60 is considered **very high**.

Table 8.3: Risk ranking summary of *Escherichia coli* O157:H7 contamination of South African grown plums locally and following export to the United Kingdom and the European Union for the low and high susceptible consumers, taking the effectiveness of the post-processing control system into account

Risk Ranger Question[a]	United Kingdom Risk				European Risk			
	Controlled Post-processing Control System		Poorly Controlled Post-processing Control System		Controlled Post-processing Control System		Poorly Controlled Post-processing Control System	
	Low	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
Q1	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Q2	General	Extremely	General	Extremely	General	Extremely	General	Extremely
Q3	Few	Few	Few	Few	Few	Few	Few	Few
Q4	75%	75%	75%	75%	25%	25%	25%	25%
Q5	62300000	114 766	62300000	114 766	425418981	2300000	425418981	2300000
Q6	Rare	Rare	Rare	Rare	Rare	Rare	Rare	Rare
Q7	Usually eliminates	Usually eliminates	Usually eliminates	Usually eliminates	Usually eliminates	Usually eliminates	Usually eliminates	Usually eliminates
Q8	No	No	No	No	No	No	No	No
Q9	Well controlled	Well controlled	Not controlled	Not controlled	Well controlled	Well controlled	Not controlled	Not controlled
Q10	100x	10x	100x	10x	100x	10x	100x	10x
Q11	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction
Probability of illness[c]	8.22E-11	1.64E-07	8.22E-10	1.64E-06	8.22E-11	1.64E-07	8.22E-10	1.64E-06
Total predicted illnesses[d]	1.4E+00	5.16E-03	1.4E+01	5.16E-02	3.19E+00	3.45E-02	3.19E+01	3.45E-01
Risk ranking value	30	32	36	38	28	29	33	35
Risk Ranking[e]	**Low**	**Medium**	**Medium**	**Medium**	**Medium**	**Low**	**Medium**	**Medium**

[a]:Q1, hazard severity; Q2, susceptibility of population; Q3, product consumption frequency; Q4, proportion of population consuming that product; Q5, size of consuming population; Q6, probability of contamination of raw product; Q7, effect of processing on the hazard, Q8, potential for recontamination of product after processing; Q9, effectiveness of post-processing control system; Q10, increase of hazard needed to cause illness; Q11, preparation of product before eating. [b]: High risk and low risk population groups, [c]: per day per consumer of interest, [d]: Per annum in population of interest, [e]: Risk <32 is considered **low**, risk between 32 and 48 is considered **medium**, risk between 48 and 60 is considered **high** and risk >60 is considered **very high**.

For both the UK and EU general population the *L. monocytogenes*-plum pairing is considered a low risk (UK Risk ranking: 25 and EU Risk ranking: 22), with well controlled post-processing control systems (Table 8.5). For the more susceptible population, the risk is considered medium in the UK (Risk ranking: 32) but low for the EU (Risk ranking: 29), with well controlled post-processing control systems (Table 8.5). Similarly, when the post-processing control system is not functioning optimally then the risk following consumption of a potentially contaminated *L. monocytogenes* plum was considered low in the UK and EU general population (Risk ranking: 30 and 28) scenario (Table 8.5). For extremely susceptible population in the UK and EU the risk increased to a medium risk (Risk ranking: 38 and 35) (Table 8.5). According to the risk assessment tool, only 0.14 and 0.39 cases of illness will result following the consumption of potentially *L. monocytogenes* contaminated plums in the general population of the UK and EU, respectively when the post-processing control system is in place. Predicted illnesses that may result in the UK and EU in the extremely susceptible population are 0.0051 and 0.0345 cases of illness will result in extremely susceptible portions of the population in the UK and EU, respectively (Table 8.5). If the post-processing control system is not adequately in place and controlled a ten-fold increase in illnesses can be predicted (Table 8.5).

The *Salmonella* Typhimurium-peach pairing is a low risk pairing for the general and vulnerable population of UK (Risk ranking: 28 and 28) and EU (Risk ranking: 24 and 24) when a well-controlled post-processing system is in place (Table 8.6). In the UK only 4.67 and 0.001 illnesses could potentially result in the general population and extremely susceptible population following the consumption of a potentially contaminated peach. Additionally, only 6.38 and 0.05 illnesses could result for the EU general and extremely susceptible populations (Table 8.6). When a lack of effective post-processing control system is considered the risk ranking changes to a medium risk for the UK consumers (Risk ranking: 33 and 34) but remains a low risk for the EU population (Risk ranking: 29 and 30) (Table 8.6). When the post-processing control system fails the number of predicted illnesses increases tenfold for the UK and EU general and vulnerable population categories (Table 8.6).

Table 8.4: Risk ranking summary of *Listeria monocytogenes* contamination of South African grown peaches locally and following export to the United Kingdom and the European Union for the low and high susceptible consumers, taking the effectiveness of the post-processing control system into account

Risk Ranger Question[a]	United Kingdom Risk				European Risk			
	Controlled Post-processing Control System		Poorly Controlled Post-processing Control System		Controlled Post-processing Control System		Poorly Controlled Post-processing Control System	
	Low	High	Low	High	Low	High	Low	High
Q1	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Q2	General	Extremely	General	Extremely	General	Extremely	General	Extremely
Q3	Few	Few	Few	Few	Few	Few	Few	Few
Q4	25%	25%	25%	25%	5%	5%	5%	5%
Q5	62300000	114766	62300000	114766	425418981	2300000	425418981	2300000
Q6	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent
Q7	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction
Q8	No	No	No	No	No	No	No	No
Q9	Well Controlled	Well Controlled	No control	No control	Well Controlled	Well Controlled	No control	No control
Q10	1000x	10x	1000x	10x	1000x	10x	1000x	10x
Q11	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction
Probability of illness[c]	4.11E-09	8.22E-05	4.11E-08	8.22E-04	4.11E-09	8.22E-05	4.11E-08	8.22E-04
Total predicted illnesses[d]	2.34E+01	8.61E-01	2.34E+02	8.61E+00	3.19E+01	3.45E+00	3.19E+02	3.45E+01
Risk ranking value	37	45	43	51	41	41	39	47
Risk Ranking[e]	**Medium**	**Medium**	**Medium**	**High**	**Medium**	**Medium**	**Medium**	**Medium**

[a]:Q1, hazard severity; Q2, susceptibility of population; Q3, product consumption frequency; Q4, proportion of population consuming that product; Q5, size of consuming population; Q6, probability of contamination of raw product; Q7, effect of processing on the hazard, Q8, potential for recontamination of product after processing; Q9, effectiveness of post-processing control system; Q10, increase of hazard needed to cause illness; Q11, preparation of product before eating. [b]: High risk and low risk population groups, [c]: per day per consumer of interest, [d]: Per annum in population of interest, [e]: Risk <32 is considered **low**, risk between 32 and 48 is considered **medium**, risk between 48 and 60 is considered **high** and risk >60 is considered **very high**.

Table 8.5: Risk ranking summary of *Listeria monocytogenes* contamination of South African grown plums locally and following export to the United Kingdom and the European Union for the low and high susceptible consumers, taking the effectiveness of the post-processing control system into account

Risk Ranger Question[a]	United Kingdom Risk				European Risk			
	Controlled Post-processing Control System		Poorly Controlled Post-processing Control System		Controlled Post-processing Control System		Poorly Controlled Post-processing Control System	
	Low	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
Q1	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Q2	General	Extremely	General	Extremely	General	Extremely	General	Extremely
Q3	Few	Few	Few	Few	Few	Few	Few	Few
Q4	75%	75%	75%	75%	25%	25%	25%	25%
Q5	62300000	114766	62300000	114766	425418981	2300000	425418981	2300000
Q6	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent
Q7	Usually Eliminates	Usually Eliminates	Usually Eliminates	Usually Eliminates	Usually Eliminates	Usually Eliminates	Usually Eliminates	Usually Eliminates
Q8	No	No	No	No	No	No	No	No
Q9	Well Controlled	Well Controlled	No control	No control	Well Controlled	Well Controlled	No control	No control
Q10	10 000x	1 00x	10 000x	1 00x	10 000x	100x	10 000x	100x
Q11	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction
Probability of illness[c]	8.22E-12	1.64E-07	8.22E-11	1.64E-06	8.22E-12	1.64E-07	8.22E-11	1.64E-06
Total predicted illnesses[d]	1.40E-01	5.16E-03	1.40E+00	5.16E-02	3.19E-01	3.45E-02	3.19E+00	3.45E-01
Risk ranking value	25	32	30	38	22	29	28	35
Risk Ranking[e]	**Low**	**Medium**	**Low**	**Medium**	**Low**	**Low**	**Low**	**Medium**

[a]:Q1, hazard severity; Q2, susceptibility of population; Q3, product consumption frequency; Q4, proportion of population consuming that product; Q5, size of consuming population; Q6, probability of contamination of raw product; Q7, effect of processing on the hazard, Q8, potential for recontamination of product after processing; Q9, effectiveness of post-processing control system; Q10, increase of hazard needed to cause illness; Q11, preparation of product before eating. [b]: High risk and low risk population groups, [c]: per day per consumer of interest, [d]: Per annum in population of interest, [e]: Risk <32 is considered **low**, risk between 32 and 48 is considered **medium**, risk between 48 and 60 is considered **high** and risk >60 is considered **very high**.

Table 8.6: Risk ranking summary of *Salmonella* Typhimurium contamination of South African grown peaches locally and following export to the United Kingdom and the European Union for the low and high susceptible consumers, taking the effectiveness of the post-processing control system into account

Risk Ranger Question[a]	United Kingdom Risk				European Risk			
	Controlled Post-processing Control System		Poorly Controlled Post-processing Control System		Controlled Post-processing Control System		Poorly Controlled Post-processing Control System	
	Low	High	Low	High	Low	High	Low	High
Q1	Mild	Mild	Mild	Mild	Mild	Mild	Mild	Mild
Q2	General	Extremely	General	Extremely	General	Extremely	General	Extremely
Q3	Few	Few	Few	Few	Few	Few	Few	Few
Q4	25%	25%	25%	25%	5%	5%	5%	5%
Q5	62300000	114766	62300000	114766	425418981	2300000	425418981	2300000
Q6	Other: 0.01%	Other: 0.01%	Other: 0.01%	Other: 0.01%	Other: 0.01%	Other: 0.01%	Other: 0.01%	Other: 0.01%
Q7	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect
Q8	No	No	No	No	No	No	No	No
Q9	Well Controlled	Well Controlled	Not controlled	Not controlled	Well Controlled	Well Controlled	Not controlled	Not controlled
Q10	100x	15x	100x	15x	100x	15x	100x	15x
Q11	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction
Probability of illness	8.22E-10	1.10E-06	8.22E-09	1.10E-05	8.22E-10	1.10E-06	8.22E-09	1.10E-05
Total predicted illnesses	4.67E+00	1.15E-02	4.67E+01	1.15E-01	6.38E+00	4.60E-02	6.38E+01	4.60E-01
Risk ranking value	28	28	33	34	24	24	29	30
Risk Ranking	**Low**	**Low**	**Medium**	**Medium**	**Low**	**Low**	**Low**	**Low**

[a]:Q1, hazard severity; Q2, susceptibility of population; Q3, product consumption frequency; Q4, proportion of population consuming that product; Q5, size of consuming population; Q6, probability of contamination of raw product; Q7, effect of processing on the hazard, Q8, potential for recontamination of product after processing; Q9, effectiveness of post-processing control system; Q10, increase of hazard needed to cause illness; Q11, preparation of product before eating. [b]: High risk and low risk population groups, [c]: per day per consumer of interest, [d]: Per annum in population of interest, [e]: Risk <32 is considered **low**, risk between 32 and 48 is considered **medium**, risk between 48 and 60 is considered **high** and risk >60 is considered **very high**.

Table 8.7: Risk ranking summary of *Salmonella* Typhimurium contamination of South African grown plums locally and following export to the United Kingdom and the European Union for the low and high susceptible consumers, taking the effectiveness of the post-processing control system into account

Risk Ranger Question[a]	United Kingdom Risk				European Risk			
	Controlled Post-processing Control System		Poorly Controlled Post-processing Control System		Controlled Post-processing Control System		Poorly Controlled Post-processing Control System	
	Low	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
Q1	Mild	Mild	Mild	Mild	Mild	Mild	Mild	Mild
Q2	General	Extremely	General	Extremely	General	Extremely	General	Extremely
Q3	Few	Few	Few	Few	Few	Few	Few	Few
Q4	75%	75%	75%	75%	25%	25%	25%	25%
Q5	62300000	114766	62300000	114766	425418981	2300000	425418981	2300000
Q6	Other: 0.01%	Other: 0.01%	Other: 0.01%	Other: 0.01%	Other: 0.01%	Other: 0.01%	Other: 0.01%	Other: 0.01%
Q7	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction
Q8	No	No	No	No	No	No	No	No
Q9	Well Controlled	Well Controlled	Not controlled	Not controlled	Well Controlled	Well Controlled	Not controlled	Not controlled
Q10	1 000x	1 50x	1 000x	1 50x	1 000x	1 50x	1 000x	1 50x
Q11	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction
Probability of illness[c]	4.11E-11	5.48E-08	4.11E-10	5.48E-07	4.11E-11	5.48E-08	4.11E-10	5.48E-07
Total predicted illnesses[d]	7.01E-01	1.73E-03	7.01E+00	1.72E-02	1.60E+00	1.15E-02	1.6E+01	1.15E-01
Risk ranking value	23	24	29	29	29	21	26	27
Risk Ranking[e]	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**

[a]:Q1, hazard severity; Q2, susceptibility of population; Q3, product consumption frequency; Q4, proportion of population consuming that product; Q5, size of consuming population; Q6, probability of contamination of raw product; Q7, effect of processing on the hazard, Q8, potential for recontamination of product after processing; Q9, effectiveness of post-processing control system; Q10, increase of hazard needed to cause illness; Q11, preparation of product before eating. [b]: High risk and low risk population groups, [c]: per day per consumer of interest, [d]: Per annum in population of interest, [e]: Risk <32 is considered **low**, risk between 32 and 48 is considered **medium**, risk between 48 and 60 is considered **high** and risk >60 is considered **very high**.

The *Salmonella* Typhimurium-plum pairing risk for the general and extremely susceptible consumer groups within the UK and EU populations were all low. The UK risk ranking was 23 and 29 for the general population and 24 and 29 for the vulnerable population. The EU risk ranking was 20 and 26 for the general population and 21 and 27 for the vulnerable population) (Table 8.7). Very few illnesses were predicted to occur following the consumption of plums potentially contaminated with *Salmonella* Typhimurium within the general (0.701 illnesses) and extremely susceptible (0.0017 illnesses) population of UK, when the post-processing control system was functioning effectively (Table 8.7). For the general population of the EU only 1.6 illnesses were predicted and for the extremely susceptible only 0.015 illnesses were predicted, when well controlled post-processing control systems were implemented (Table 8.7). A tenfold increase in predicted illnesses was observed for the UK and EU when the post-processing control system was not implemented and effectively managed (Table 8.6).

South African grown peaches exported to the UK and being consumed by the general (Risk ranking: 22) and susceptible (Risk ranking: 18) population were considered to be of low risk when a well-controlled post-processing control system is implemented (Table 8.8). Only 4.67 and 0.0017 cases of illness due to *S. aureus* contamination of SA grown peaches being consumed in the UK, was estimated (Table 8.8). Even when the post-processing control system is not effectively managed the risk to the general population and extremely susceptible population remains low (Risk ranking: 28 and 24) (Table 8.8). With the increase in the risk ranking value, there was a corresponding tenfold increase in the estimated number of predicted illnesses (Table 8.8).

In the EU the risk of the *S. aureus*- peach pairing was considered to be low, under all conditions and by all the consumer groups (Table 8.8). A low risk was determined for the general population (Risk ranking: 18 and 24) as well as for the extremely susceptible population (Risk ranking: 14 and 20), in respective of the effectiveness of the post-processing control system (Table 8.8). It was predicted that in the general population, only 6.38 and 63.8 cases of illness could possibly result following the consumption of peaches by the entire population, when the post-processing system was well controlled or not controlled, respectively (Table 8.8). Only, 0.007 and 0.069 possible cases of illness could result for the susceptible population when a well-controlled and not controlled post-processing control system, respectively (Table 8.8).

The risk to the general and extremely susceptible (Risk ranking: 25 and 21) UK consumers when the post-processing control system is well controlled was predicted to be a low risk. If

the post-processing system is not effectively maintained and managed then the risk to consumers was predicted also to be low for the general and extremely susceptible population (Risk ranking: 30 and 26) (Table 8.9). Only 14 and 0.005 estimated illnesses could result following the consumption of SA plums that may possibly be contaminated with *S. aureus* for the general and extremely susceptible population with a well-controlled post-processing control system, respectively (Table 8.9). A tenfold increase in predicted number of illnesses was determined if the post-processing control system was not functioning effectively (Table 8.9).

A similar trend to the UK's risk was observed with the risk to the EU population. For the general and vulnerable EU population the risk to the consumer was considered low irrespective of the efficiency of the post-processing control system. When the post-processing control system is well controlled the predicted risk for the general and susceptible population was considered to be low with a risk ranking of 22 and 18, respectively. If the post-processing control system was not well managed then the predicted risk ranking increased to 28 and 24, respectively for the general and susceptible population. Only an estimated 31.9 illnesses would result with a well-controlled post-processing system implemented and 319.0 illnesses if the post-processing control system was not controlled (Table 8.9). In the vulnerable population consuming potentially contaminated plums only 0.035 and 0.35 cases of illness were predicted if the post-processing control system was and was not efficiently controlled, respectively (Table 8.9).

Table 8.8: Risk ranking summary of *Staphylococcus aureus* contamination of South African grown peaches locally and following export to the United Kingdom and the European Union for the low and high susceptible consumers, taking the effectiveness of the post-processing control system into account

Risk Ranger Question[a]	United Kingdom Risk				European Risk			
	Controlled Post-processing Control System		Poorly Controlled Post-processing Control System		Controlled Post-processing Control System		Poorly Controlled Post-processing Control System	
	Low	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
Q1	Minor	Minor	Minor	Minor	Minor	Minor	Minor	Minor
Q2	General	Extremely	General	Extremely	General	Extremely	General	Extremely
Q3	Few	Few	Few	Few	Few	Few	Few	Few
Q4	25%	25%	25%	25%	5%	5%	5%	5%
Q5	62300000	114766	62300000	114766	425418981	2300000	425418981	2300000
Q6	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent	Infrequent
Q7	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction
Q8	Yes, minor	Yes, minor	Yes, minor	Yes, minor	Yes, minor	Yes, minor	Yes, minor	Yes, minor
Q9	Well Controlled	Well Controlled	Not controlled	Not controlled	Well Controlled	Well Controlled	Not controlled	Not controlled
Q10	10 000x	10 000x	10 000x	10 000x	10 000x	10 000x	10 000x	10 000x
Q11	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction
Probability of illness[c]	8.22E-10	1.64E-07	8.22E-09	1.64E-06	8.22E-10	1.64E-07	8.22E-09	1.64E-06
Total predicted illnesses[d]	4.67E+00	1.72E-03	4.67E+01	1.72E-02	6.38E+00	6.9E-03	6.38E+01	6.90E-02
Risk ranking value	22	18	28	24	118	14	24	20
Risk Ranking[e]	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**

[a]:Q1, hazard severity; Q2, susceptibility of population; Q3, product consumption frequency; Q4, proportion of population consuming that product; Q5, size of consuming population; Q6, probability of contamination of raw product; Q7, effect of processing on the hazard, Q8, potential for recontamination of product after processing; Q9, effectiveness of post-processing control system; Q10, increase of hazard needed to cause illness; Q11, preparation of product before eating. [b]: High risk and low risk population groups, [c]: per day per consumer of interest, [d]: Per annum in population of interest, [e]: Risk <32 is considered **low**, risk between 32 and 48 is considered **medium**, risk between 48 and 60 is considered **high** and risk >60 is considered **very high**.

Table 8.9: Risk ranking summary of *Staphylococcus aureus* contamination of South African grown plums locally and following export to the United Kingdom and the European Union for the low and high susceptible consumers, taking the effectiveness of the post-processing control system into account

Risk Ranger Question[a]	United Kingdom Risk				European Risk			
	Controlled Post-processing Control System		Poorly Controlled Post-processing Control System		Controlled Post-processing Control System		Poorly Controlled Post-processing Control System	
	Low	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
Q1	Minor	Minor	Minor	Minor	Minor	Minor	Minor	Minor
Q2	General	Extremely	General	Extremely	General	Extremely	General	Extremely
Q3	Few	Few	Few	Few	Few	Few	Few	Few
Q4	75%	75%	75%	75%	25%	25%	25%	25%
Q5	62300000	114766	62300000	114766	425418981	2300000	425418981	2300000
Q6	Rare	Rare	Rare	Rare	Rare	Rare	Rare	Rare
Q7	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction	Slight reduction
Q8	Yes, minor	Yes, minor	Yes, minor	Yes, minor	Yes, minor	Yes, minor	Yes, minor	Yes, minor
Q9	Well Controlled	Well Controlled	Not controlled	Not controlled	Well Controlled	Well Controlled	Not controlled	Not controlled
Q10	10 000x	10 000x	10 000x	10 000x	10 000x	10 000x	10 000x	10 000x
Q11	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction	1 log reduction
Probability of illness[c]	8.22E-10	1.64E-07	8.22E-09	1.64E-06	8.22E-10	1.64E-07	8.22E-09	1.64E-06
Total predicted illnesses[d]	1.4E+01	5.16E-03	1.40E-02	5.16E-02	3.19E01	3.45E-02	3.19E+02	3.45E-01
Risk ranking value	25	21	30	26	22	18	28	24
Risk Ranking[e]	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**

[a]:Q1, hazard severity; Q2, susceptibility of population; Q3, product consumption frequency; Q4, proportion of population consuming that product; Q5, size of consuming population; Q6, probability of contamination of raw product; Q7, effect of processing on the hazard, Q8, potential for recontamination of product after processing; Q9, effectiveness of post-processing control system; Q10, increase of hazard needed to cause illness; Q11, preparation of product before eating. [b]: High risk and low risk population groups, [c]: per day per consumer of interest, [d]: Per annum in population of interest, [e]: Risk <32 is considered **low**, risk between 32 and 48 is considered **medium**, risk between 48 and 60 is considered **high** and risk >60 is considered **very high**.

4. Discussion

This is the very first report where the Risk Ranger (Ross and Sumner, 2002) was used to characterise the risk in the fresh fruit supply chain as a semi-quantitative risk assessment tool. It is important to note that the Risk Ranger was not developed as an exact and precise tool in terms of predicted cases of illnesses. The Risk Ranger estimates should be interpreted as estimates and cannot emulate the richness of a full quantitative risk assessment. This is a useful tool as a prelude or type of "pilot study" to focus industry research, by screening the foodborne risks and identifying those that might require more rigorous assessment (Sumner and Ross, 2005).

According to Sumner and Ross (2005), a risk "reality check" is required by comparing the risk rankings to epidemiological data available, because the number of predicted illnesses cannot be compared to the actual events occurring. Not one foodborne illness according to the literature and the authors' knowledge has been linked to the consumption of peaches or plums. In this study the risk of hazard-peach and hazard-plum pairings resulted in a low to medium risk depending on the consuming population groups.

Peaches and plums are mainly exported to the UK and EU, with only 25% of and 75% of people from the UK and 5% and 25% of Europeans consuming peaches and plums respectively and only a few times per year. International consumers are at low to medium risk of contracting a foodborne illness following the consumption of peaches and plums grown in SA. The risk posed to consumers is mainly dependent on the detection rate of the pathogens on fruit, the effect of the export chain and the effectiveness of the post-processing control system.

Escherichia coli O157:H7 is the second most important in terms of possible risk and predicted number of illnesses. *Escherichia coli* O157:H7 is considered a moderate pathogen because illness caused by this pathogen often requires medical treatment (Mataragas *et al.*, 2008). In addition, *E. coli* had sufficiently decreasing titres following a simulated peach and plum export chain and could be considered that the titres are "usually eliminated" following the export chain (Chapter 7). *Escherichia coli* O157:H7 titres on peaches decrease following one day on peaches at room temperature and never recover during the 21 day export chain at the various temperatures that are required during the export chain (Chapter 7). On plums, titres decrease following a combination of five days at the ultra-low temperature of -0.5^{o}C and seven days at 7.5^{o}C (required for ripening) all required for exporting plums and titres recover following incubation at 4^{o}C but

then decrease again following one day incubation at 21°C (Chapter 7). *Escherichia coli* O157:H7 was only detected on 1.6% of all previously tested peaches (Chapter 4). Therefore, the risk posed following the consumption of a peach to the 5% of EU population consuming peaches was considered to be low and to the larger 25% of UK population consuming peaches was considered medium. The risk posed to the 75% of EU population consuming plums and the 25% of UK population consuming plums was considered to be low to medium. The reason for the lower risk for plums versus the peaches was due to the lower detection rate of *E. coli* O157:H7 from plums (not detected). Therefore, even though more international consumers consume plums than peaches the risk to the plum consumers if lower due to the lower contamination rate of plums (Chapter 4 and Chapter 5) and the reduction in titres when moving through the cold chain (Chapter 7). In addition if fruit are exported with a poorly controlled and managed post-processing control system then the risk increases because the abuse of the temperature scheme of the post-processing control system will no longer allow the elimination of the pathogen in the export chain.

Listeria monocytogenes is a moderate foodborne pathogen like *E. coli* O157:H7 which normally results in medical treatment following infection (Mataragas *et al.*, 2008). Recently, *L. monocytogenes* was determined to be present on peaches at a rate of 2.5% (Chapter 4) and 2.0% on plums (Chapter 5) and was therefore determined to be infrequently detected (1% according to Risk Ranger) from peaches and plums entering the export chain. *Listeria monocytogenes* is able to survive freezing temperatures (Adams and Moss, 2000). The peach export chain is shorter than the plum export chain and therefore during the peach export chain *L. monocytogenes* survived the cold temperatures but during the peach export chain *L. monocytogenes* more effectively recovered from the cold temperatures than during the plum export chain (Chapter 7). In addition, peaches have an increased surface area therefore creating niches where the pathogen was able to survive (Chapter 7). All these factors lead to the prediction that *L. monocytogenes* is a medium to high risk for peach consuming populations and a low to medium risk to plum consuming populations.

Interestingly, *Salmonella* Typhimurium was also better able to survive the export chain on peaches when compared to plums. *Salmonella* Typhimurium was not once detected from the 1 087 total samples collected from all four farms investigated (Chapter 4 and Chapter 5), therefore the detection rate was determined as other 0.01%. Due to the low detection rate and the ability of *Salmonella* Typhimurium to survive better on peaches through the export chain the risk to consumers consuming plums is lower than the risk to peach

consumers. With possible *Salmonella* Typhimurium contamination of peaches and plums the efficiency of the post-processing control system only affects the risk to peach consumers, because a poorly managed control system might lead to the increase of possibly present *Salmonella* Typhimurium.

Staphylococcus aureus is a minor foodborne pathogen that is an indicator of poor personal hygiene (Adams and Moss, 2000). Recently, *S. aureus* was rarely detected on plums and infrequently on peaches (Chapter 4 and Chapter 5). *Staphylococcus aureus* titres were determined to show a slight reduction on both peaches and plums when artificially inoculated onto the fruit and following the simulation of an international export chain (Chapter 7). All international consumers are at low risk following the consumption of possibly contaminated peaches or plums.

Listeria monocytogenes illnesses that were predicted to occur in the general population of the UK and EU (23 and 31.9 illnesses) were higher when compared to predicted cases of *E. coli* O157:H7. Low numbers of *E. coli* O157:H7 illness was predicted for general consumer in the UK, where only 4.67 predicted illnesses following the consumption of peaches and only 1.4 illnesses were predicted to occur following the consumption of plums. In the EU only 6.38 predicted illnesses and 3.19 illnesses were predicted following the consumption of peaches or plums, respectively. The predicted number of illnesses that may result following the consumption of peaches was higher than following the consumption of plums. Predicted illnesses following the consumption of peaches and plums possibly contaminated with *S. aureus* were in the similar range than *E. coli* O157:H7, but these illnesses are minor in comparison. Predicted *Salmonella* Typhimurium illnesses were the least due to the low detection rate of the pathogen on raw produce. Commonly fewer illnesses were predicted for the susceptible HIV population in the UK and EU due to the smaller population considered.

The post-processing control system is an important consideration. We assumed in this study that the export control system was a stringent post-processing control system and therefore was well controlled. It was observed that if the post-processing control system for some reason was to fail the risk increased on average by 5 or 6 risk ranking points, therefore in some cases increasing from a low risk to a medium risk or from a medium to high risk. The post-processing control system is very important to manage to decrease the risk. In the subsequent Chapter (Chapter 9), research will focus on the horticultural safety management system. By ensuring that the horticultural safety management system

is well managed the risk to consumers will also decrease because the prevalence of contamination should decrease.

In conclusion, *Salmonella* Typhimurium and *S. aureus* are the lowest risk pathogens on peaches and plums when the post-processing control system is well maintained, managed and implemented. *Escherichia coli* O157:H7 poses a low to medium risk to consumers in the UK and EU, in order to decrease the risk to consumers the on-farm horticultural safety management system needs to be improved to decrease the number of peaches and plums contaminated with the pathogen. There should be a zero tolerance for the presence of *E. coli* O157:H7 and *L. monocytogenes* on produce as well as within the environment. *Listeria monocytogenes* poses a low to high risk to consumers.

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Chapter 9

The Evaluation of a Diagnostic Tool to Assess the Horticultural Safety Management System of Primary Production of Two Peach and Plum Farms in South Africa

Stacey Collignon and Lise Korsten

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Abstract

In the horticultural industry where fruit and vegetables are produced, it is important to ensure that produce is cultivated and packed within an environment that will ensure food safety. In order to attain produce safety a food safety management system needs to be implemented. A diagnostic tool was developed to self-assess and eventually improve the horticultural safety management system (HSMS) of primary producers. The aim of this study was to evaluate this self-diagnosis tool for the South Africa peach and plum producers. The diagnostic tool aims to assess contextual factors, core safety control activities, core assurance activities and food safety performance of the primary producers. Once the food safety profile of the primary production system has been determined producers can be advised as to how to improve the HSMS, therefore aiming to assure a safer product. Using the self-diagnostic tool, it was determined that all four primary producers assessed had a high risk contextual situation wherein peaches and plums are being produced (in terms of product, process, organisational and chain characteristics). Therefore, an advanced HSMS will be required to assure food safety systems compliance. However the assessment of the HSMS reveals an overall basic level of food safety compliance. The results obtained in this study indicate that all primary producers would likely need to implement similar improvements in the HSMS. Taking this diagnostic tool into consideration main changes would therefore be required including more regular testing for the presence of foodborne contamination and maximum pesticide residue levels. The quality of the in-orchard water supply should be improved to prevent product contamination. The tool also indicates that better sanitation of facilities and equipment would be required to improve the food safety assurance system. This self-diagnostic tool if used in the South African context would need to be tailored to the local situation and made more adaptive to include a more risk and science based approach since increased testing does not necessarily underscore food safety assurance.

1. Introduction

The safety of fresh produce has become an important and relevant aspect to be considered in international trade and for the domestic food industry. The new age global drive to healthier eating habits has led to an increase in consumption of fruit and vegetables. This has led to a greater demand for fresh produce throughout the year, even when locally unavailable (Meng and Doyle, 2002). The greater demand for fresh produce has thus resulted in an increase in production and imported volumes (Jacxsens *et al.*, 2010). Retailers increasingly procure from foreign countries to ensure that variety and volumes are made available throughout the year to the customer (Dillard and German, 2000). This increase in demand leads to an increased food safety assurance demand of fresh produce which is often sourced from developing countries with varying levels of food safety management practices. Produce exported from these countries are thought to pose a greater threat due to the perceived unhygienic production and packing practices. Developing countries are also perceived to use potentially contaminated water for irrigation, pesticide spraying and sometimes even in the washing of fresh produce postharvest. This resulted in the implementation of voluntary good agricultural practices and food safety standards for producers, to ensure and assure food safety. In order to achieve this level of assurance, it is essential that all role players comply with a basic food safety management system as the product moves from the farm to the table (Ropkins and Beck, 2000).

Internationally, there are various voluntary quality assurance standards or private schemes that are implemented in different agricultural and food industries. These standards or schemes are normally required by retailers prior to procuring fresh produce or food products. Pre-requisite programmes, such as good hygiene practices and good agricultural practices, need to be implemented prior to certification. The challenge for producers is thus the effective implementation of these pre-requisite programmes within a broader quality assurance program evolving to a higher level or overall food safety management systems. Such a broader food safety management system is therefore implemented to increase assurance, reduce risk and to ultimately prevent outbreaks of foodborne illnesses (Jacxsens *et al.* 2009; Luning *et al.*, 2006 and Tsalo *et al.*, 2007). A food safety management system should thus ensure that the food product is not prone to pathogen contamination, within an environment that allow for growth or survival and which requires an adequate administrative framework and an ability to ensure that decisions on food safety management can be made independently. Luning and Marcelis (2007), defined a food safety management system as a company specific system that allows

control and assurance activities that realise and guarantee food safety. The control activities within a Horticultural Safety Management System (HSMS) provide for the identification of areas of improvement to ensure that the product and processes are controlled. The assurance activities on the other hand ensure that the necessary performance evaluations and the organisation of the required changes in systems are recorded to reflect overall compliance.

The performance of a food safety management system can only be determined if assessed at various levels. The challenge therefore is how to assess the food safety management system of producers, Luning *et al.* (2011) developed a diagnostic tool that allows food producers to assess the food safety management system according to certain specific situational factors that will influence the food safety output of a company's food safety management system. Once the assessment has been completed the diagnostic tool can provide an idea of the level of risk (Luning *et al.,* 2011). Following the challenges of the food safety management system, a primary producer can implement the necessary changes to decrease the riskiness of the specific situation and so improve the performance of the food safety management system. If the food safety management system is working optimally, then the final products will have a lower contamination level and less variation in the inoculum load (Luning *et al.*, 2011). It is important to note, however, that this diagnostic tool does not translate to the actual microbiological performance of a producer but only gives an indication of the theoretical effectiveness of the food safety management system. The microbial performance of a producer still needs to be assessed using a Microbiological Assessment Scheme, which is based on the results of the diagnostic tool.

The aim of this study was to evaluate the diagnostic tool to assess the HSMS within the South Africa fresh produce industry, using peach and plum producers as a case study. Results of the diagnostic tool will be used in the overall food safety output assessment of two plum and peach farms. Proposed improvements in the HSMS application in the South African situation has been included in this chapter.

2. Methodology

The diagnostic tool was evaluated by undertaking an actual "diagnosis" on two plum (Plum Farm 1 and 2) and two peach farms (Peach Farm 1 and 2). These farms represent classical examples of commercial production systems in South Africa. An assessment of the HSMS was conducted to determine the level of food safety output within the primary

production system. The plum and peach farms (described in Chapter 4 and Chapter 5) were assessed based on predefined questions. The questions were divided into five parts each one dealing with a specific factor or activity. Part 0 is the introductory questions dealing with the basics of the farm. Part I addresses the contextual factors. Part II addresses the assessment of the core safety control activities and Part III address the assessment of the core assurance activities. Lastly, Part IV addresses the assessment of the food safety performance. The information was collected by the main author through interviews with farm managers from all four farms under the following sections (section 2.1 to 2.3) and the data was expressed in spider web diagrams within the specific sections.

2.1. Assessment of context characteristics

The contextual situation was assessed on characteristics of the product, process, organisation, and the environment. The product and process characteristics give insight in the sensitivity towards pathogen contamination, growth or survival, whereas organisational characteristics reflect the administrative conditions in the company or the farm. The characteristics of the chain environment give an indication about the organisation's dependency, which affects its room for decision-making on food safety (management) issues.

For each characteristic, three contextual situations have been described to differentiate the level of company riskiness to decision making in the HSMS, *i.e.* low, medium and high risk. Situation 1 (low risk) is for product and process characteristics typically associated with low initial contamination, poor growth conditions, survival potential and lack of vulnerability (for cross contamination and unexpected problems). For organisational characteristics it refers to aspects like high workforce quality, supportive organisational structures, specific information systems (to support decision making). For chain environment characteristics it is associated with low dependency and lack of vulnerability to food safety problems. Situation 2 (medium risk) is for product and process characteristics typically associated with potentially vulnerable situations or critical for contamination (with *i.e.* pathogens), growth and survival potential of microorganisms. For organisational characteristics it refers to aspects like constraints associated with the workforce, restricted organisational structures and restricted information systems. For chain environment characteristics it is associated with restricted dependency and potential vulnerability to food safety problems. Situation 3 (high risk) is for product and process characteristics typically associated with highly vulnerable or critical for contamination, growth, and/or survival of pathogenic microorganisms situations. For organisational

characteristics it refers to aspects like low workforce quality, lack of organisational structures, lack of information systems to support HSMS decisions. For chain environment characteristics it is associated with highly dependent and highly vulnerable situations to food safety problems.

For the contextual evaluation a more coloured spider web diagram is associated with a more risky contextual situation. The more risky the contextual situation the more easily a food safety problem may arise, which will put higher demands on the HSMS.

To obtain a more general picture of the contextual situation, an overall score was assigned to each facet. For this purpose an average score of the contextual situation for the product and process characteristic and for the organisation and chain characteristics, was calculated. The overall score was assigned by interpreting the mean score as 1 (low risk) if the mean score of characteristics is 1 to 1.2, score 1-2 (low-medium risk) if mean score of characteristics is 1.3 to 1.7, score 2 (medium risk) if mean score of characteristics is 1.8 to 2.2, score 2-3 (medium-high risk) if mean score of characteristics is 2.3 to 2.7 and score 3 (high risk) if mean score of characteristics is 2.8 to 3.0.

2.2. Assessment of horticulture safety management system

The core control and assurance activities of two peach and two plum farms were assessed using the HSMS diagnostic instrument. Safety control aims at keeping product properties, production processes, and human processes between certain acceptable tolerances, whereas the objective of assurance is to control the quality management system and to provide evidence and confidence to stakeholders about meeting the quality requirements. It is assumed that control and assurance activities executed on a higher level are more predictable and more likely to result in a desired safety outcome. Core control activities are those activities that considerably contribute to the realisation of a safe product by evaluation of the performance of both technological and human processes and taking corrective actions when necessary. The control activities are distinguished in three different strategies *i.e.* preventive measures, intervention processes, and monitoring. Both the design and the actual operation of these core control strategies are assessed. Core assurance activities are those activities that considerably contribute to ensure that the products are safe and comply with stakeholder demands, by setting requirements on the quality system, evaluating its performance, and organising necessary changes.

For each core activity three different situations have been described, which represent different levels of compliance. These levels give an indication about how the core activities are designed and are functioning in practice, low level (Situation 1) represents a situation when an activity is absent, not applicable, or unknown; basic level (Situation 2) relates with lack of scientific evidence, use of company experience/history, variable, unknown, unpredictable, based on common materials/equipment; average level (Situation 3) corresponds with best practice knowledge/equipment, sometimes variable, not always predictable, based on generic information/guidelines for the product sector; and advanced level (Situation 4) is associated with scientifically underpinned (accurate, complete), stable, predictable, and tailored for the specific food production situation.

Similarly, three different situations have been described for the assurance activities, low level (Situation 1) represents a situation when an activity is absent, not applicable, or unknown; basic level (Situation 2) corresponds to situations when changes in the HSMS are initiated by (stakeholder) problems (reactive); validation by using historical experience/data, no independent judgement; verification done by only checking presence of procedures/records, parameter settings, scarcely reported, documented; documentation is not structured, ad-hoc, no access external sources, average level (Situation 3) is associated with actively acting on stakeholder requirements; validation done by the use of expert knowledge/regulatory documents, (internal) expert judgement; verification performed by additional analysis procedures, records, regular reporting (expert/internal reports) and documentation that is structured, kept up to date, de-centrally organised, access via authorised persons, restricted external sources and advanced level (Situation 4) is associated with pro-actively acting on stakeholder requirements; validation is using specific scientific sources, own test/trials, judgement by external experts; verification additional analysis, and actual performance measuring, comprehensive reporting/documentation; documentation is structured, kept-up-to date, centrally organised, available for all, access external information sources.

A more coloured food safety control and assurance activities spider web diagram is associated with a higher level of food safety control and assurance activities.

To obtain an overall picture of the HSMS activities, overall levels were assigned by averaging the activity levels. The overall level that was assigned was interpreted by the mean score, level 1 (basic) if mean score of activities is 1 to 2.2, level 1-2 (basic-average) if mean of score of activities is 2.3 to 2.7, level 2 (average) if mean score of activities is

2.8 to 3.2, level 2-3 (average-advanced) if mean score of activities is 3.3 to 3.7 and level 3 (advanced) if mean score of activities is 3.8 to 4.0.

2.3. Assessment of food safety output of the system

In addition an assessment of the food safety output of the HSMS was performed. A "diagnosis" was done by using several indicators with corresponding grids based on existing internal and external company information (*i.e.* information from audits, complaints, non-conformities, samplings). Two types of indicators were defined for assessment. The first types of indicators were selected to assess how independent experts appreciated the specific HSMS (*i.e.* external food safety output). The second type of indicators was based on information from samplings and non-conformity registration within the farm/company (internal food safety output).

Again different situations were described to give an indication about the food safety output of the HSMS. Situation 1 (No indication) refers to absent, not present or not conducted, Situation 2 (Poor performance) is associated with aspects like ad-hoc sampling, minimal criteria used for HSMS evaluation, and having various food safety problems due to different challenges in the HSMS, Situation 3 (Moderate performance) is referring to regular sampling, several criteria used for HSMS evaluation, and having restricted food safety problems mainly due to one (Restricted) type of problem in the HSMS and Situation 4 (Good performance) is referring to a systematic evaluation of the HSMS using specific criteria and having no safety problems.

A more coloured food safety output spider web diagram is associated with a higher level of food safety output.

To obtain an overall food safety output, overall levels were assigned by averaging the activity levels and the scores were the same as section 2.2.

3. Results

Results presented in this section are the actual diagnostic tool results and the results of the evaluation of this diagnostic tool are discussed in more detail in the discussion section.

All farms in this assessment according to the product and processing characteristics can be grouped at a medium to high risk (2-3) level (Figure 9.1; Appendix A). Factors that contribute to this medium to high risk cannot be influenced by the farm management systems but could be considered generic for the industry in South Africa. The initial materials are all classified as a medium risk according to the microbiological, pesticide and mycotoxin contamination risk due to the growing conditions that the trees undergo prior to planting in the orchards (Figure 9.1; Appendix A). The final products from all farms are also considered to be of medium risk in terms of potential microbiological contamination. Peach Farm 1 and Plum Farm 2 are certified organic farms and therefore the risk that produce will exceed the pesticide maximum residue level (MRL) for these two farms is low, whereas Peach Farm 2 and Plum Farm 1 use conventional production systems. The final product of Peach Farm 2 and Plum Farm 1 can be considered to be of a medium risk in terms of potential to exceed the MRL of pesticide on the final product (Figure 9.1; Appendix A). However, if correct procedures are followed with only registered pesticides being used and if produce withholding times are strictly adhered to then this assumption will not be valid. These two farms are Global GAP certified, this provides some indication that these measures should be in place. All four farms are considered according to the HSMS tool to be rated as high risk in terms of the production system because of open production systems that use soil, the sub-tropical climate that the fruit trees are growing in and due to the common association of possible contaminated water supply and the use of surface water (Figure 9.1, Appendix A). However, such a generalisation is not valid since certain generic assumptions cannot be made.

On the other hand the organisational and chain characteristics are more easily controlled. The overall score for the organisation and chain characteristics for all four farms evaluated was considered of a medium-high (2-3) risk (Figure 9.2 A to D). All farms evaluated do not have staff specifically appointed to manage the food safety system. Personnel do not have official training in food safety and this was a contributing factor to the higher level of risk. Both peach farms have a low turnover of employees with occasional temporary workers. These factors were rated as contributing to lowering the level when compared to the plum farms that have a higher worker turnover rate. All workers from all four farms have no specific higher education, only basic food safety training and limited food safety experience with no specified language ability and are not involved in the improvement of the HSMS (Appendix A). The two peach farms and Plum Farm 2 have no formal food safety policy or team, with few to no procedures and no food safety information in the standard information system (Figure 9.2 A to D; Appendix A). Plum Farm 1 has a general

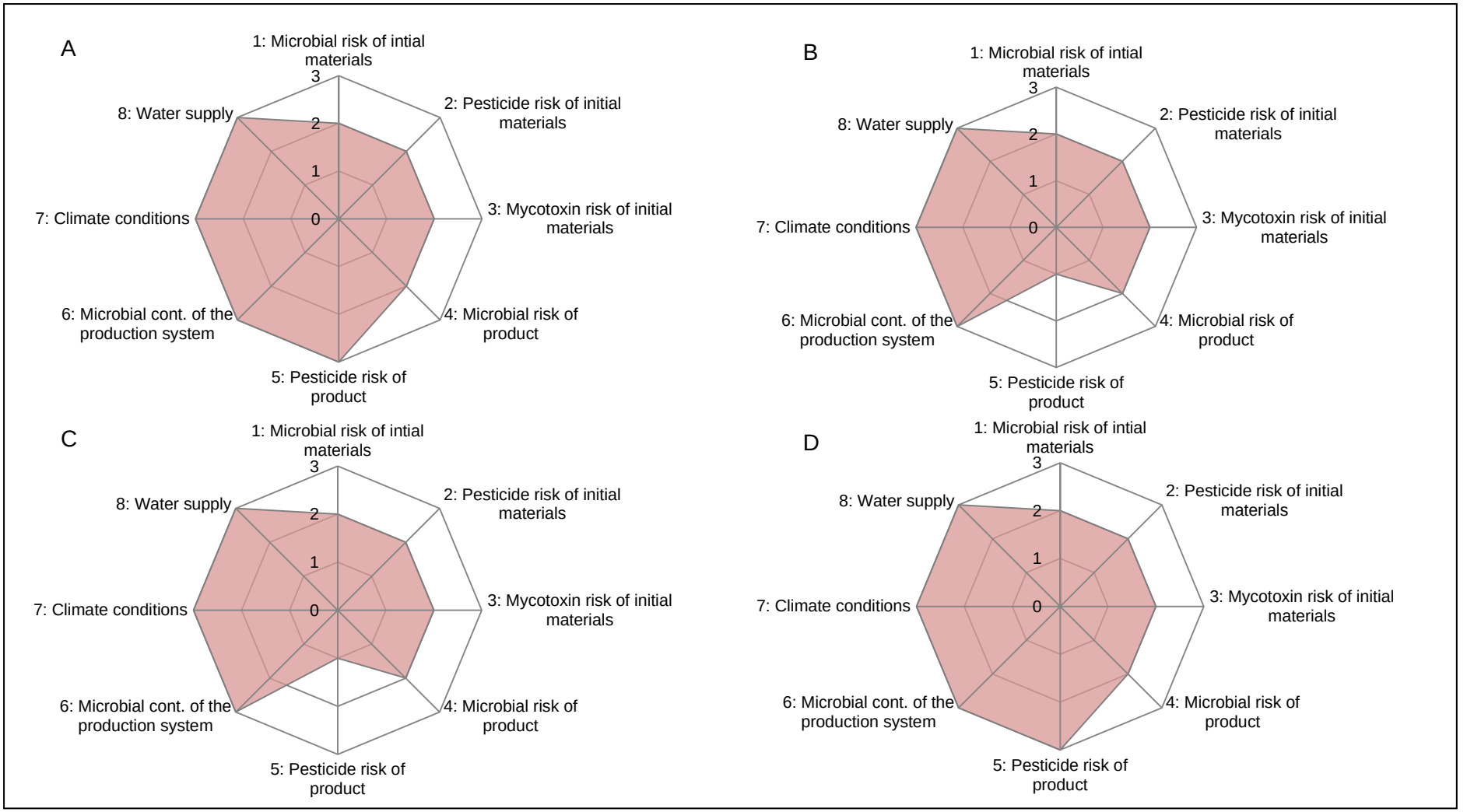

Figure 9.1: Assessment of product and process characteristics from Peach Farm 1 (A), Peach Farm 2 (B), Plum Farm 1 (C) and Plum Farm 2 (D).

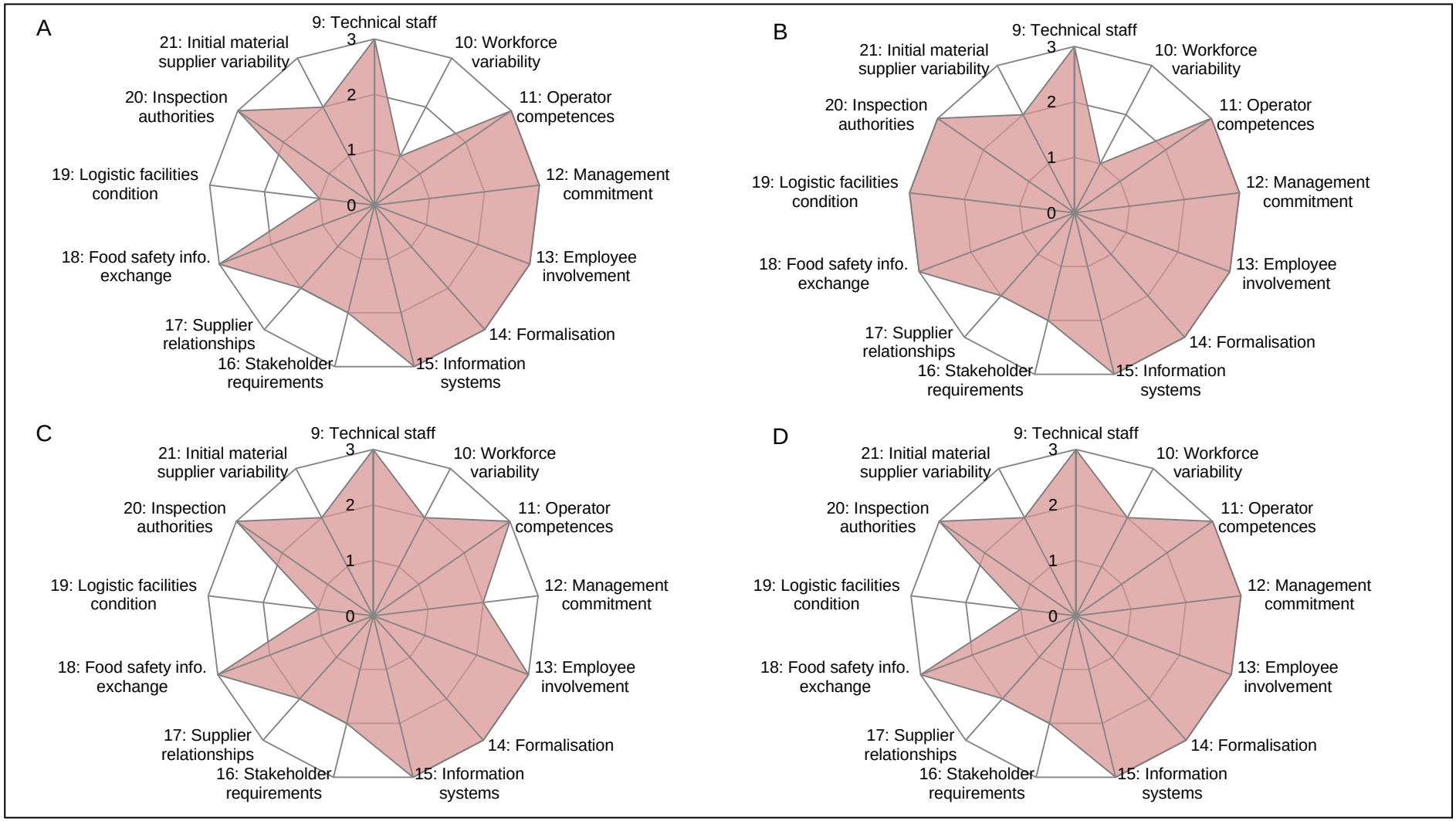

Figure 9.2: Assessment of organisation and chain characteristics from Peach Farm 1 (A), Peach Farm 2 (B), Plum Farm 1 (C) and Plum Farm 2 (D).

food safety policy, introduced by an external party but have no food safety procedures or information systems.

Chain characteristics for all farms contributed to the overall medium-high risk score with the lack of information exchange with suppliers and relationships based on specifications, and especially with the lack of external support and implementation of food safety legislation. In South Africa, implementation of an HSMS is not a regulation and therefore it is not necessary for the government health and food safety officers to control or inspect primary production. Third party inspectors and auditors working for a certification body determine the level of compliance of the voluntary standards or private schemes and food safety management systems according to stakeholders' (retailers) requirements. Peach Farm 1 and the two plum farms have strictly controlled logistical facilities, with conditions modified for specific types of products, for Peach Farm 2 this is however not the case. All four farms could invest into a dedicated quality assurance manager or team with on-site or collaborative expertise in food safety to ensure that all aspects are positively addressed adding to food safety assurance. An additional factor that will improve the overall riskiness of the organisation characteristics is investment into training current staff in food safety programmes.

Figure 9.3 shows the spider web diagrams of control activities (preventive measures, intervention process, monitoring system) and Figure 9.4 show the actual operation of the activities. The overall score of the preventative measures, intervention process and monitoring system activities are 2.1 (basic) for Peach Farm 1 (Figure 9.3A), 1.6 (basic) for Peach Farm 2 (Figure 9.3B), 1.8 (basic) for Plum Farm 1 (Figure 9.3 C) and 2.2 (basic) for Plum Farm 2 (Figure 9.3D) (Appendix A).

Peach Farm 1 and Plum Farm 1 and 2 all have equipment, facilities and packaging equipment that were not specifically designed for the product but do meet the basic hygiene requirements. Equipment is maintained but is this is not well documented. Peach Farm 2, does not use equipment on the farm, as packaging is conducted by a retail consortium (Figure 9.3 and Figure 9.4). The riskiness of the preventative measures and intervention processes can be improved by changing equipment, upgrading facilities and improving packaging equipment that are specific for the product and will contribute to potential prevention of product contamination.

A compliant aspect for Peach Farm 1 and both plum farms is the storage facility. These storage facilities are designed and managed on an advanced level (Figure 9.3 and Figure

9.4; Appendix A). Storage on Peach Farm 2, on the other hand is not specific for the product, automatically controlled and deviations in the environment are not systematically analysed (Figure 9.3 and Figure 9.4; Appendix A). Most preventative measures on Peach Farm 1, Plum Farm 1 and Plum Farm 2 are designed according to best available practices and knowledge. Personal hygiene requirements, supplier control, fertiliser program and irrigation methods are developed using generic information/guidelines for the product sector. Peach Farm 2 has standard requirements for personal hygiene with instructions but have no specific supplier control requirements (Figure 9.3B). Peach Farm 1 and Plum Farm 2 also use registered pesticides based on qualified expert advice (Figure 9.3 A and 9.3 D; Appendix A). Peach Farm 2 uses the optimal industry recognised fertiliser program and irrigation method for the product (Figure 9.3B). Peach Farm 2 and Plum Farm 1 are organic farms and therefore no conventional chemical pesticides are used. Peach Farm 1, Plum Farm 1 and Plum Farm 2 all use sanitation programs that are not specific to the equipment or facility but rather a general sanitation program with common cleaning agents and Peach Farm 2 has no specific sanitation program. The sanitation programme implemented on the four farms can be adjusted for different equipment and facilities with alternative cleaning agents that are specific and tested within the farms environment.

None of the farms exhibit control on incoming goods in terms of testing the food safety of the incoming goods. Water is only tested on an ad hoc basis, therefore “water control” is considered to be at a basic level. In addition surface water is also used that is prone to contamination (Chapter 4 and Chapter 5). Water should also be tested by an accredited laboratory that has validated sampling protocols, minimize sample storage time prior to analysis and effectively test water on a routine basis, this will increase the level of HSMS. No physical intervention processes exist that can be used to reduce the possible microbial loads (Figure 9.3 and Figure 9.4).

No assessment of possible microbial contamination is conducted. According the this diagnostic tool it is important that primary producers need to regularly send the final product for microbiological testing and have the correct judgement criteria for the microbiological test results. Only Peach Farm 1 and Plum Farm 4 test for compliance to the required maximum residue pesticide levels. These primary producers rely on the Perishable Product Export Control Board to use sampling plans based on either in-house knowledge with no information available on the sensitivity of the analytical equipment (Figure 9.3 A, Figure 9.3 D, Figure 9.4 A) or based on common practice in the sector (Figure 9.3 C). The other two farms are organic farms and do not use pesticide and therefore there is no need to test for pesticide residues. No corrective action is however

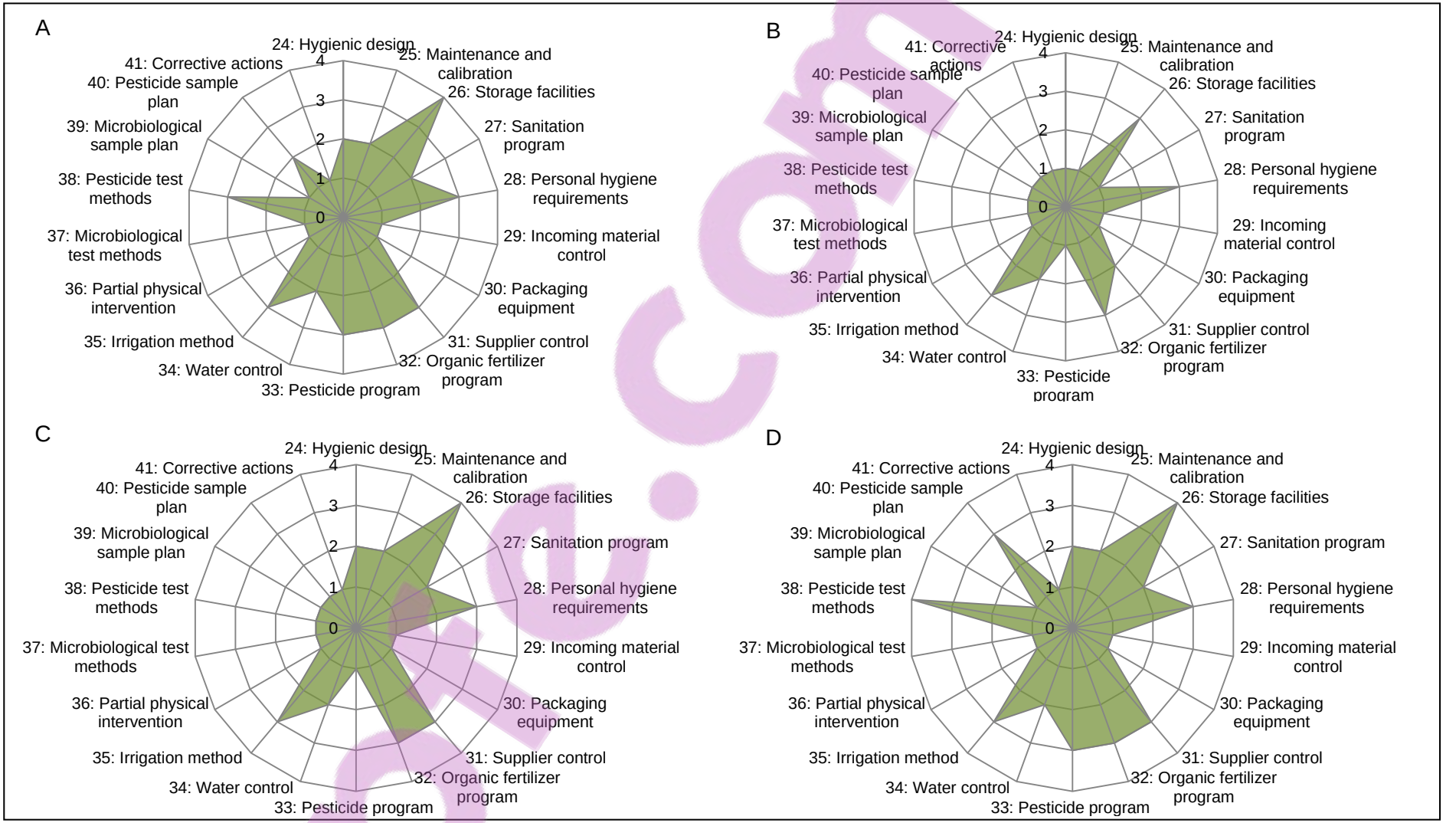

Figure 9.3: Assessment of preventative measures, intervention process design and monitoring system from Peach Farm 1 (A), Peach Farm 2 (B), Plum Farm 1 (C) and Plum Farm 2 (D).

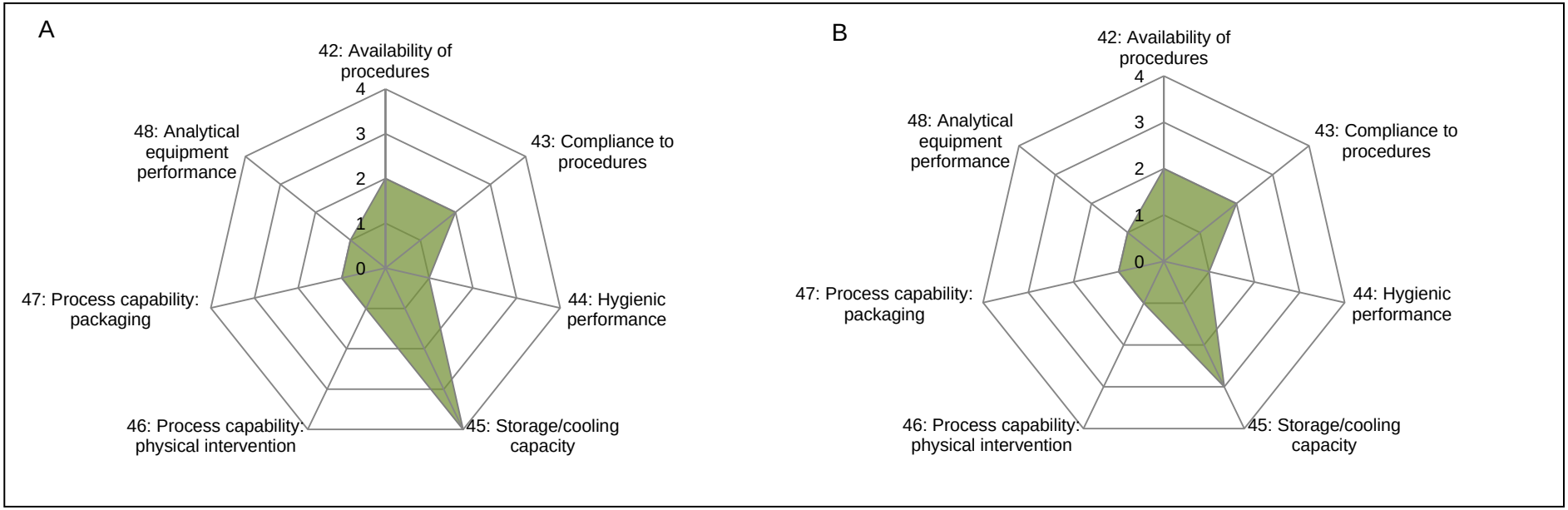

Figure 9.4: Assessment of Core Assurance Activities, setting system requirements activities, validation activities, verification activities and documentation and record-keeping to support food assurance for Peach Farm 1, Plum Farm 1 and Plum Farm 2 (A) and Peach Farm 2 (B).

defined for any of the four farms, in the event of pesticide or microbial contamination. In addition, the indicators for actual operation show that procedures are not up-to-date and not available on location. In addition, the majority of the agricultural workers are not aware of the procedures and execute the tasks according to their own insight.

The assessment of the core assurance activities including the activities of setting the HSMS, validation, verification and documentation revealed that all four farms had a basic score. In Figure 9.5 and Appendix A it can be seen that documentation and record keeping are done at an average level (situation 3), meaning that they are structured, kept up to date, de-centrally organised, accessed via authorised persons, and using restricted external sources. The rest of the assurance activities are at a basic level (situation 2), except the validation of physical interventions which is not conducted on any of the farms. To improve the assurance activities they need to be validated by external and independent experts. Changes in the HSMS are initiated by requirements of the stakeholders or problems (reactive), farmers need to take a more proactive approach to improve the level of the HSMS. Validation is done by using historical experience/data, and is not done by independent people. Verification is performed by checking presence of procedures/records, parameter settings, and scarcely reported, documented, to improve the level of verification the performance needs to be analysed and confirmation of performance by actual testing on a regular basis.

The overall score of the food safety output of the HSMS of Peach Farm 1 (score: 2.8), Plum Farm 1 (score: 2.6) and Plum Farm 2 (score: 2.4) is poor-moderate and poor for Peach Farm 2 (score: 1.3) (Figure 9.6, Appendix A). Peach Farm 1, Plum Farm 1 and Plum Farm 2 have a moderate external judgement of the HSMS due to the fact that the farm is audited by one accredited certification body with no major remarks on specific aspects of their implemented food safety management system (Figure 9.6 A, C and D, Appendix A). Customer complaints regarding poor hygiene, microbiological and chemical safety aspects have not been received by Peach Farm 1 and Plum Farm 1. Peach Farm 2 and Plum Farm 2 have no way to register complaints and therefore the complaints are unknown. A critical requirement for all food safety management systems is the establishment of a customer complaint system and to ensure that it is effectively implemented. None of the farms have received complaints regarding the visual quality of the fruit (Figure 9.6, Appendix A). The internal food safety output is judged at poor for Peach Farm 2 and Plum Farm 1 as no information from sampling can be retrieved as no sampling is done (Figure 9.6, Appendix A). Peach Farm 1 and Plum Farm 2 do conduct pesticide testing therefore their overall internal food safety output is a bit higher but still

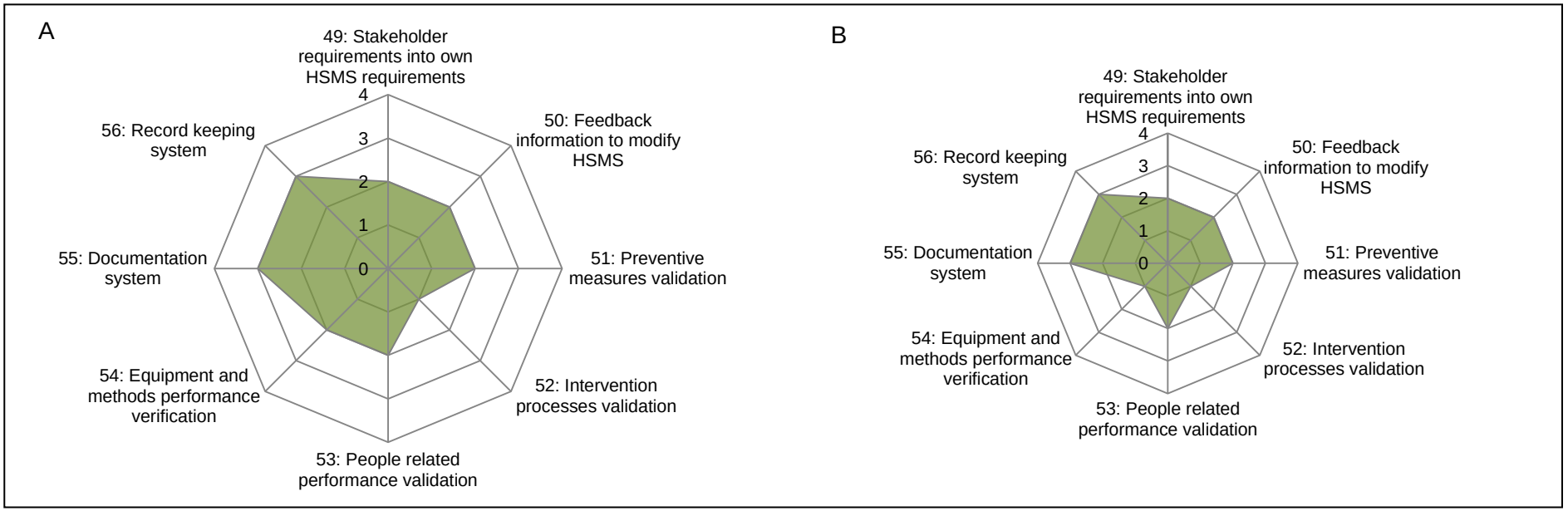

Figure 9.5: Assessment of Core Assurance Activities, setting system requirements activities, validation activities, verification activities and documentation and record-keeping to support food assurance for Peach Farm 1, Plum Farm 1 and Plum Farm 2 (A) and Peach Farm 2 (B).

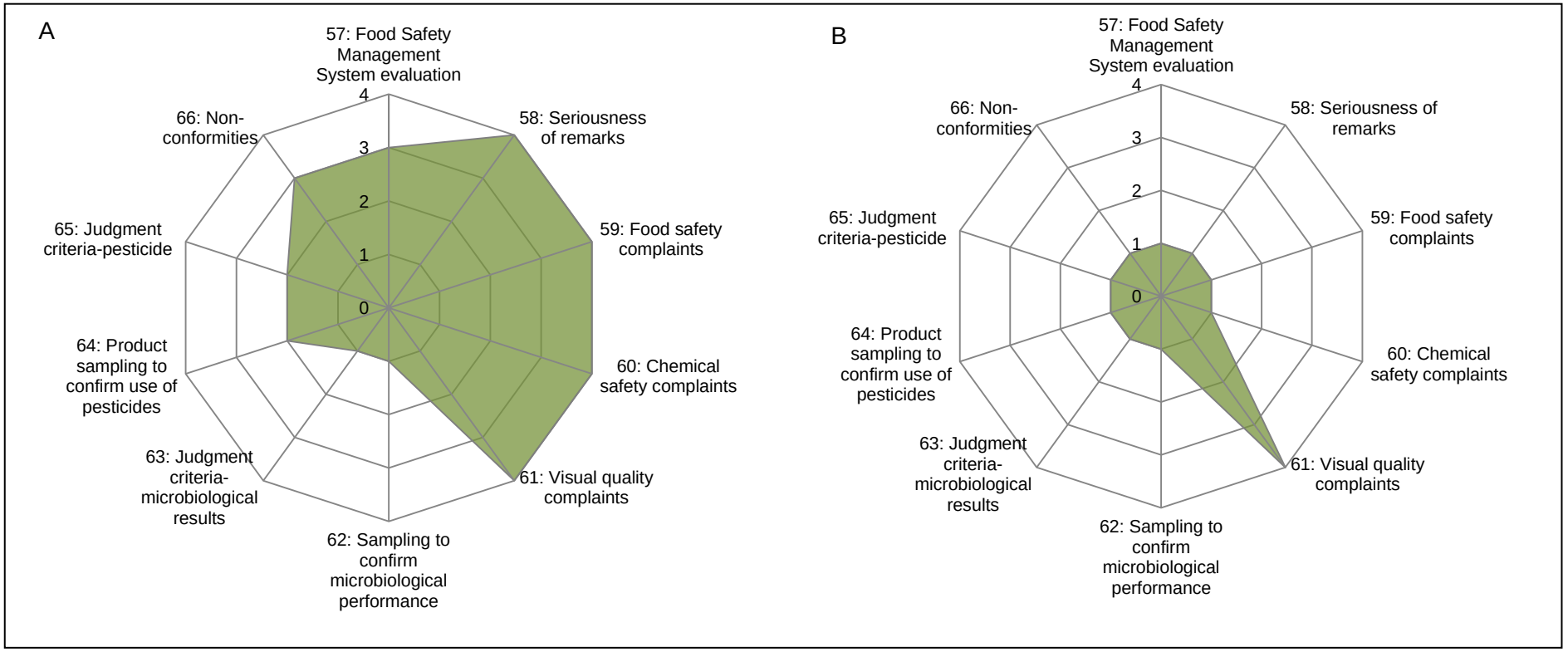

Figure 9.6: Assessment of Food Safety Performance, External and Internal Food Safety Performance Indicators for Peach Farm 1 (A), Peach Farm 2 (B), Plum Farm 1 (C) and Plum Farm 2 (D).

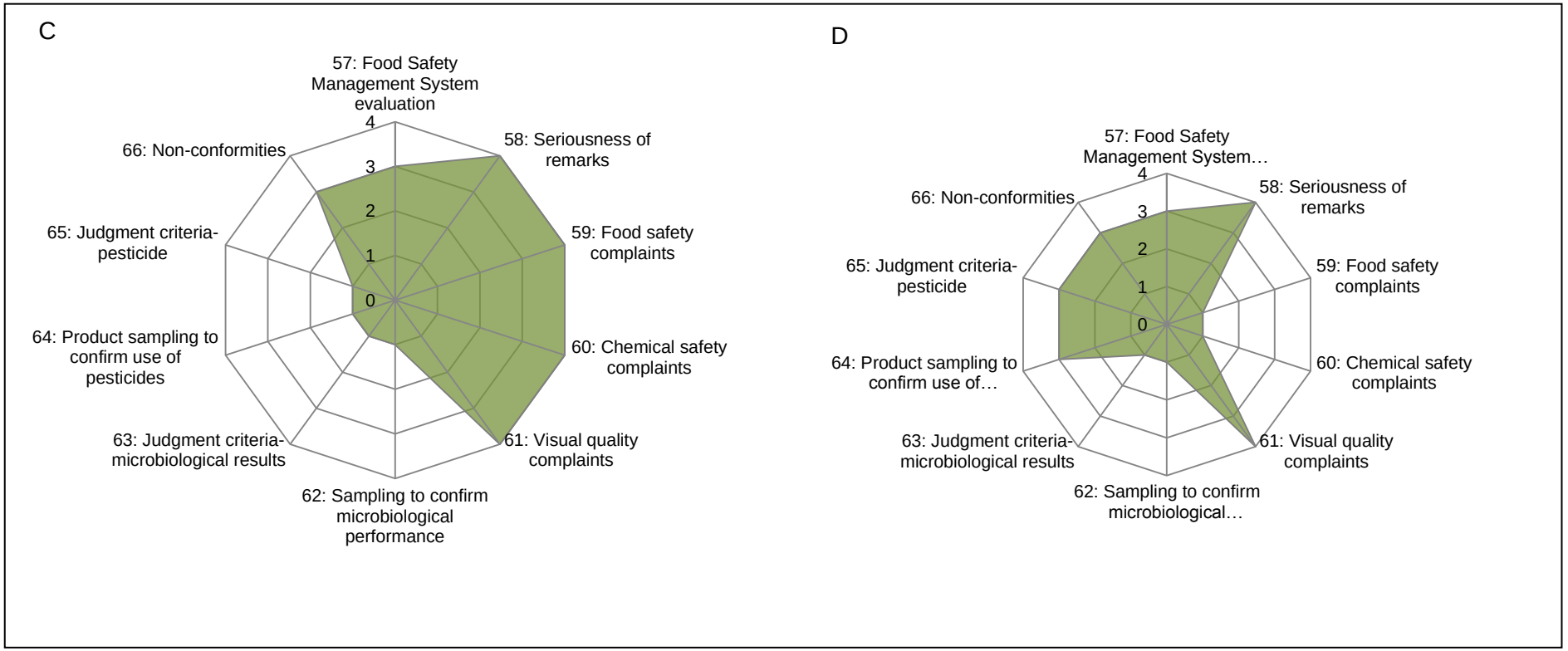

Figure 9.6 continued.

only poor and poor-moderate, respectively (Figure 9.6, Appendix A). Again the importance of testing for microbiological and pesticide residue levels is evident in the assessment of the overall food safety output. Peach Farm 2 has no non-conformance registration in terms of the final product, as no auditing takes place and Peach Farm 1, Plum Farm 1 and 2 have restricted numbers of non-conformances (Figure 9.6, Appendix A).

4. Discussion

The various contextual factors that were assessed using the HSMS diagnostic tool were aimed at assessing the potential level of risk on food safety assurance. For all farms evaluated in this assessment the product and process characteristics contributed to the medium to high risk rating. Based on the structure of the questions outlined in the diagnostic tool the water supply, tropical climate, open production system and potential contamination of initial raw materials add to the medium to high score. The assumption that some of these criteria are applicable in the South African context for primary production of stone fruit is debatable.

Water of good quality is becoming a scarce commodity worldwide (Kirby et al., 2003). Produce can come into contact with polluted water through irrigation or pesticide application. This direct exposure of fresh produce with polluted water during production could potentially allow enough contact time for the attachment of waterborne pathogens. Water is not only a risk in terms of irrigation, but also in terms of pesticide application. Pesticides reconstituted in potentially contaminated water have previously been shown to be stable environments for *Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes* (Guan *et al.* 2001; Coghan, 2000) and could therefore be a source of contamination (Ng *et al.*, 2005). Water has previously been shown to be a source of persistent foodborne pathogens and represents a potential risk for contamination of an agricultural crop. The evidence, however, of a direct link between produce contaminated at the primary producer and foodborne illness is scarce (Suslow *et al.*, 2003). The link has therefore not been made between poor water quality and foodborne disease outbreaks. Drip irrigation is known to reduce the level of risk of contamination of fruit hanging on trees (Suslow *et al.*, 2003) except for low hanging fruit within the drip line. Realistically many countries do not have excellent quality water which is used for irrigation and it is unrealistic to expect that irrigation water should be potable water. In South Africa the water quality guidelines for irrigation state that irrigation water used for fruit trees should contain less than 1000 *E. coli* counts/100ml (Department of Water Affairs and Forestry, 1996). If in the unlikely event

fruit do become contaminated, the foodborne pathogens need to survive the various hurdle steps during packing, cold storage, transportation and distribution. We recommend that the focus on water supply should only be a considered as a potential risk in the post-harvest usage of water on fruit and for hygiene and sanitation practices. Water is used in the postharvest environment in peach and plum packhouses for cooling systems, sanitation and personal hygiene practices. Water that is used in the packhouse for sanitation and hygiene is required to be potable water (Kirby *et al.*, 2003). Venter and September (2006), however found that clean and moderately contaminated water had no effect on the reduction of the microbial load on heavily contaminated hands if hands were air dried, but the main decontaminating factor was the use of paper towels to dry hands. The hand washing procedure should therefore also be taking into consideration in the diagnostic tool as well as the quality of water used for hand washing.

Foodborne pathogens are able to survive in all climatic zones but a tropical climate is more conducive to the growth and survival of pathogens, by increasing the replication cycles of pathogens. Winfield and Groisman (2003) stated that *Escherichia coli* is able to survive in tropical climates due to the high nutrient concentrations available in the ecosystem, the constant warm air, soil and water temperatures, providing an ideal habitat for survival, growth and proliferation. However, South Africa with its variation in climatic zones cannot be considered a tropical climate and peach and peach production is mostly from subtropical climatic zones. An important consideration that was excluded in the assessment tool was UV exposure and countries closer to the poles do have higher levels of daily UV radiation and sunshine hours. The impact of UV on field grown fruit has not been determined and should in future be considered before a general statement on in field survival is made.

Secondary contamination of fruit from other sources like birds, farm animals (like cattle and game) and insects are increased in an open production system because there is no barrier to protect the crop. The open production system of peach and plum farms in South Africa therefore adds to the medium to high risk category of the product and process characteristics. However, farming systems in South Africa is on a larger scale than most other countries and vast distances between farms and cities are common. In addition, integrated farming is not common place although game is sporadically observed in certain orchards if farming is done in close proximity to nature conservation areas. The likelihood of bird droppings should be considered low as well as exposure to animals. However in this study it was found that geese were closely associated with production areas and did contribute to surface contamination (Chapter 5). Following the first season of

investigation these geese were removed and surface contamination of fruit with *E. coli* O157:H7 decreased.

According to this diagnostic tool an important control activity that was determined as required was the testing of the final product for foodborne pathogens and pesticide MRLs. Realistically the primary producer is unable to send samples to accredited laboratories to test for foodborne pathogens since end point inspection is considered an obsolete approach to food safety assurance and that a systems approach to food safety management is considered more effective. If procedures are in place and no previous microbial risk has been shown it is unnecessary to require microbial sampling and analysis. Currently it was found that only peaches from Peach Farm 1 and plums from Plum Farm 1 were contaminated with a foodborne pathogen (Chapter 4 and Chapter 5). The natural protection that prevent microorganisms from entering the fruit allows the product to be rated as a medium risk category and not a high risk category. In addition the export conditions that peaches and plums undergo do not allow the proliferation of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* if the conditions of export and transportation are well controlled (Chapter 7). The risk to international consumers of peaches and plums from South Africa is low to medium (Chapter 8). In South Africa the PPECB are responsible for testing fresh produce for pesticide MRLs prior to leaving the farm and entering the relevant markets. The PPECB is also responsible for the sampling plan employed which is based on Agricultural Product Standards Act No. 119 of 1990. The responsibility for sampling, testing, maintaining the analytical equipment and interpreting the results does not lie with the primary producers and is therefore not applicable in this context. It is our recommendation that the testing of mycotoxins, pesticide residues and foodborne pathogens be excluded from the assessment criteria since it is not relevant in this context.

The storage facility of the primary producer is important in terms of hygiene and temperature management of the environment and the impact it has on the fruit and contaminant in terms of proliferation. All the farms assessed in this study have good storage facilities therefore decreasing the risk. At fluctuating and non-stable temperatures the titre of foodborne pathogens can increase on the surface of peaches and plums (Chapter 6). The effectively and certified controlled temperatures throughout the export chain will not allow the persistence of low concentrations of possible pathogens to survive (Collignon and Korsten, 2010; Chapter 7).

Poor sanitary practices and personal hygiene of workers could potentially contribute to contamination of produce during growing, harvesting and packing (Brackett, 1999; Doyle, 2000; James, 2006). Poor personal hygiene of workers handling produce has been responsible for over 40% of source identified produce-related outbreaks (Bean and Griffin, 1990). Karitsky *et al.* (1984), found that 74% of the different Norwalklike virus outbreaks from 1983 to 1991 were associated with infected food handlers. Hygiene of workers and facility sanitation is therefore an important control activity that can prevent unnecessary contamination of fresh produce (Centers for Disease Control and Prevention, 1999). All four primary producers in this study were rated as having good hygiene practices and a moderate sanitation programme implemented. Despite these perceived good and moderate practices implemented hand and contact surface contamination was found in this study (Chapter 4 and Chapter 5). Primary producers should therefore focus their food safety management systems on training staff in the correct hygiene and sanitation practices and procedures required to assure compliance in personal hygiene and environmental sanitation.

Farm workers are responsible for good hygiene practices and for proper sanitation on the farm. Training of food handlers should be considered a primary preventative control measure (Soon *et al.*, 2010). Training and experience of farm workers is an important aspect to ensure that these procedures are followed and implemented correctly. In a survey conducted in 1996, 33.5% of farm workers in South Africa are seasonal or casual with the remaining 66.5% regular workers (Anonymous, 2012). Primary producers in South Africa therefore need to train new and old staff on a seasonal basis to ensure that casual or seasonal farm workers fully understand the importance of proper hygiene and sanitation practices. The emphasis of the training should also fall on continual improvement and awareness. The Agricultural Survey done in 1996 revealed that farm workers mainly live on the farms where they work (83%) with no tap water (65%) and no toilets (80%) (Anonymous, 2012). Personal hygiene for farm workers with running potable tap water in their homes was in the minority (Anonymous, 2012), therefore demonstrating the importance of regular training in hygiene and sanitation practices (Anonymous, 2012). Farm workers have varying levels of education with 33% having no education and approximately 20% with Grade 1 to Grade 5 education and the remaining 47% having an education above Grade 5 level (Anonymous, 2012). Training of farm workers handling the produce as pickers and packers should be conducted by a third party. Training is often outsourced in South Africa but in house refresher training is often not implemented. Often primary producers do not have sufficient knowledge or time to ensure effective understanding of basic food safety principles. Ensuring that training is also done in the

workers native language is also of importance and should be considered with seasonal workers from different regions to support effective training (Soon *et al.*, 2010). Another barrier to training is a communication barrier. Chapman and Powell (2002) found that farmers' poor communication with farm workers was a barrier to the implementation of food safety programs, therefore also implying that producers were unable to properly convey the reasons for the implementation of HSMS. Clayton *et al.* (2002), also found that educating food handlers does not necessarily lead to an adaption in their food handling behaviours but mainly depends on their outlook, therefore consideration to cultural and social aspects needs to be made.

The effectiveness of a HSMS is evaluated using the food safety output of the diagnostic tool and is dependent on a number of factors. If proper record keeping is done then the HSMS will also function correctly. It has previously been found that keeping documentation up-to-date and recording all practices was identified as an implementation barrier because it is time consuming and the importance and relevance to food safety was not understood by the farmer (Chapman and Powell, 2002). In the case of commercial production in South Africa with most growers exporting and being certified to Global GAP compliance with effective documentation has become the norm.

In conclusion, the diagnosis assessment reflected that a high risk of the context requires an advanced level of the HSMS activities to achieve a good food safety output. In this study the HSMS were found to not be at an advanced level and therefore required improvement to ensure and assure food safety to consumers. To what extent this is a valid assumption given the generality of the assessment tool is debatably, therefore a more accurate assessment needs to be made with a tailored tool. The food safety output for all four farms can be improved by improving the core control and assurance activities in the system. Using this diagnostic tool determined that the main changes needed are regular testing of foodborne pathogen contamination and pesticide MRLs of the final product, microbiological testing of water and improved water control, improvement of the facility sanitation and more product specific packaging material and technologies.

Our evaluation of this diagnostic tool revealed that the system still needs to be improved to ensure industry wide adoption to ensure effective implementation as a self-assessment tool. This tool can provide an approach that can result in a more cost effective and realistic improvement matrix for primary producers. We recommend that testing of foodborne pathogens should be excluded since it remains questionable if more regular testing will contribute to improved safety assurance. Of importance is rather the proper

implementation of hygiene and sanitation regimes to ensure food safety within the primary producer environment.

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Chapter 10
General Discussion

Food safety is a critical element of food security and is directly related to ensuring adequate safe food supplies for the nation. Therefore there is a need to assure that food is free from harmful microbial, chemical and physical contaminants. A global shift in strategic thinking has resulted in a growing need for nutritious safe food as part of the food security paradigm. This has resulted in governments emphasising the need for improved public health interventions through increased consumption of fresh fruit and vegetables (Dillard and German, 2000; Meng and Doyle, 2002). In developed countries fruit and vegetables are often seasonal and limited in diversity with a distinct lack of novel and exotic produce. Improvements in distribution systems and trade flows have opened up markets for worldwide sourcing of fresh produce particularly from developing countries. In these countries food is often found year round in a wide variety of exotic fruit and vegetables. Current consumer perception is that a variety of fresh produce should be readily available throughout the year.

Food safety is an important global challenge that producers, retailers and consumers are all responsible for. Foodborne illnesses have become more prominent worldwide as lifestyle changes have resulted in altered eating patterns with increased consumption of fresh produce procured from a wider and more extensive source base. Outbreaks of illnesses caused by foodborne pathogens are unnecessary and highly preventable as increased food safety assurance systems are adopted along the food chain. Each year, millions of illnesses occur globally which are attributed to contaminated foods and has been well described by many authors (Bean and Griffin, 1990; Garcia-Villanova Ruiz *et al.*, 1987; Gerner-Smidt, 2006; Lindqvist *et al.*, 2000; Notermans and Borgdorff, 1997). Foodborne pathogens can be transmitted to food products either before or after harvest, via contaminated water sources, through poor personal hygiene or unsound environmental sanitation practices (De Roever, 1998). Throughout the supply chain there are various points at which food can become contaminated and it is up to the producer, retailer and consumer to collectively ensure food safety. Producers need to ensure that produce is safe from the farm to the retailers and up to the point of consumption. Once the responsibility of the produce is handed over to the retailer where the product gets sold as food, the retailer needs to ensure that all practices to promote food safety and prevent contamination are in place. This includes effective and informative labelling as well as in store awareness initiatives. The consumer also plays an

integral role in ensuring food safety. Once purchased the responsibility falls onto the consumer to transport, store and prepare food in a way that will prevent cross contamination or proliferation of potential contaminants.

More extended food supply chains require improved food safety assurance throughout the supply chain. The impact of retailer control and role in procurement and distribution has resulted in minimum food safety assurance standards. In addition, a more sophisticated consumer with bigger choices and increased access to information is more astute of food safety issues and basic human rights. Disease outbreaks due to foodborne illnesses are increasingly becoming newsworthy and more efficient traceability systems have allowed for rapid response and more efficient reporting. New, improved, rapid and more sensitive detection systems have also contributed to faster turnaround times for outbreak discovery and resultant product recalls. Therefore consumers are now more aware of the possible risks associated with the food that they consume and can take partial responsibility to ensure a safe food framework.

During the 2010/2011 season, 67 087 tonnes (76%) of plums produced in South Africa were exported to mostly European countries with a total value of R1 020.7 million (Hortgro, 2011a). In contrast, peach and nectarine exports equate to only R67.5 million (4%) (Hortgro, 2011b). Nonetheless, both fruit crops provide an important seasonal food source for local consumers. Fresh produce has traditionally been an important traded commodity between South Africa and the European Union. Stone fruit is the fourth most important fresh fruit exported and makes an important contribution to job security in a country with an average unemployment rate of 25% (South Africa Statistics, 2012). Approximately 10 000 and 6 700 workers are directly employed by peach and plum farms, respectively. In addition, these workers have 38 000 and 27 000 dependants, respectively (Hortgro, 2011a) making it an important industry that contribute to the socio economic stability of the country.

In order for commercial producers to retain their lucrative export market share they needed to adopt voluntary international standards that were required by purchasers of their produce. Most retailers require compliance to Global GAP certification and often additional standards such as British Retailer Consortium. Certain farmers added additional voluntary standards to differentiate their product from competitors or to obtain premium prices, such standards include for instance the European Union Organic Standard and the FairTrade label. However, to what extent the industry complies with food safety assurance standards based on sound scientific information was not clear. In this context a research project on food safety was funded by the Deciduous Fruit Producers' Trust (now Fruitgro) to determine the

level of risk associated with South African peaches and plums. Fruitgro represents the interest of all stone fruit producers in South Africa and assesses global forces and market trends to support the industry and ensure market access. In this study a framework was developed to determine the real risks, potential source of contamination and the level of risks associated with the industry. This model, although not entirely representative of the entire industry (approxiametly 1% of growers were studied), has allowed the accumulation of data to gain insight into the relevant factors influencing food safety of peaches and plums. This developed framework was based on a model system to determine the accurate and sensitive detection of four known and common foodborne pathogens and their presence in the supply chain using a newly developed molecular detection method. In addition, simulation of environmental conditions that fruit will be exposed to during production, packing, distribution and marketing was assessed using growth and survival studies in simulated supply chain systems. Furthermore, a risk assessment of primary producers using a horticultural safety management system was used to determine internal levels of compliance.

The first and one of the most important aspects in the framework, was the development of a rapid, sensitive, reliable, repeatable and more cost effective test method to screen a larger number of samples for the presence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Staphylococcus aureus* in one single step. This detection method was implemented throughout the study, therefore demonstrating the importance of an accurate, reproducible and rapid detection method. The detection method that was developed and published in a peer reviewed paper (Collignon *et al.*, 2012) allowed for equal and unbiased enrichment of all four foodborne pathogens. The use of the specific and accurate primers added to the effectiveness and reproducibility of the method described that enabled low detection levels.

With the aid of the accurate and sensitive detection method, analysis of samples from farms and a semi-quantitative risk assessment tool showed that none of the four foodborne pathogens could be considered a risk for the international consumer. The framework that was developed in this study was tested and allowed for the complete evaluation of the possible food safety risks of peaches and plums produced in South Africa.

The absence of *Salmonella* Typhimurium from all four farms sampled based on more than 1000 fruit, water and contact surface samples collected from production to packaging, rated this foodborne pathogen as a low risk, despite the pathogens ability to attach, colonise and survive on peach and plum surfaces. *In vitro* simulated studies did reveal *Salmonella* Typhimurium's ability to grow or survive in nutrient rich, nutrient poor and nutrient free

environments. This organism was also able to immediately adhere to the plum and peach surface and attach within half a minute or one hour, respectively (Collignon and Korsten, 2010). Immediate adherence demonstrates the short contact time required for contamination to take place and once adhered, the pathogen was able to survive the supply chain, but not at levels high enough that could potentially result in illness [10^3 cfu (FDA, 2009)]. This low risk was further confirmed when using the semi-quantitative risk assessment that predicted that the risk to the international consumer in the UK or EU was low. In conclusion, *Salmonella* Typhimurium cannot be regarded as a risk on peaches and plums exported from SA.

Assessing the presence, importance and potential risk associated with *L. monocytogenes* it was found in this study that the pathogen was detected on fruit and contact surfaces (crate, hand, tap, gradeline and cold room floors). This pathogen was able to either grow or survive *in vitro* simulation studies in or on nutrient rich, nutrient poor and nutrient free environments. *Listeria monocytogenes* was also able to rapidly adhere and attach to the fruit surface and was able to colonise the peach and plum fruit surface (Collignon and Korsten, 2010). Survival however, was not possible at titres high enough to cause illness [10^3 cfu (FDA, 2009)]. The semi-quantitative risk assessment also indicated that the risk to consumers was low. Therefore in conclusion, *L. monocytogenes* even though detected on a small number of fruit surfaces (2% on Peach Farm 1 and Plum Farm 1 but 0% on the other two farms) in this study the pathogen could not be considered a risk when associated with peach and plum surfaces because of the low contamination rate and the fact that the pathogen was unable to proliferate under the simulated export conditions and chances of post processing contamination were considered low.

The presence of *E. coli* O157:H7 was detected in water, on contact surfaces and on one fruit sample tested. *Escherichia coli* O157:H7 was found to be able to either survive or proliferate under nutrient rich, nutrient poor or nutrient free conditions during *in vitro* simulation studies. The attachment studies demonstrated that *E. coli* O157:H7 was able to adhere and attach onto fruit surfaces in a short period of time (60s) and were able to colonise the fruit surface and survive throughout the simulated export chain (at fluctuating temperatures of 0.5, 4 and 21^{o}C over a period of 21 or 25 days) (Collignon and Korsten, 2010). However, survival under more realistic conditions was not possible at titres that exceeded the infectious dose of the organism [10^1 cfu (FDA, 2009)]. The Risk Ranger used in this study confirmed that the level of risk with the combination of *E. coli* O157:H7 and peach or plum fruit was considered to be low because it was only found to be present on one fruit sample. *Escherichia coli* has previously been detected on a plum surface (Abdelnoor *et al.*, 1983), but *E. coli* O157:H7

has not yet been linked to an outbreak of foodborne illness with an etiology of plum or peach fruit or the processed derivative.

Although *S. aureus* was only used as an indicator of poor personal hygiene and poor facility sanitation in this study, it was found to be present in water, on fruit and contact surfaces. This organism was also found to either survive or proliferate in nutrient rich, nutrient poor and nutrient free conditions, *S. aureus* had the greatest ability to survive the nutrient free surfaces when compared to the other foodborne pathogens studied in the *in vitro* simulation study. Therefore *S. aureus* can potentially survival on workers' hands and contact surfaces for at least one week. *Staphylococcus aureus* was best able to survive on peach and plum surfaces with adhesion taking place immediately (Collignon and Korsten, 2010). Colonisation and survival was possible although titres exceeding the organisms' infectious dose [10^5 cfu (FDA, 2009)] for toxin production were not reached. Even though this organism had a greater potential to colonise and survive, the risk was still confirmed as low when the semi-quantitative risk assessment was conducted.

The assessment of the horticultural safety management system (HSMS) revealed that the hygiene and facility sanitation are important aspects that need to be improved by primary producers. This was evident throughout the model when testing the food safety framework. Future research should focus on determining the levels and events of hazard exposure of workers' hands and contact surfaces. Following the determination of the exposure, a specific hygiene and sanitation management plan that falls within a functioning HSMS should be developed, established and validated to ensure compliance with regulations. The tool can in future be used to improve an individual grower's food safety compliance level.

More generally future studies should focus on the findings of this established and tested risk assessment framework and more farms with an increased sampling size should be assessed. In addition, a full quantitative risk assessment should be conducted to assess, quantify and confirm the lack of risk associated with these four foodborne pathogens in the whole peach and plum production industry. The food safety management system in the supply chain of retailers should also be assessed to ensure that all role players are also complying and taking dual responsibility for food safety assurance.

In conclusion, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* cannot be considered a high risk in the peach and plum primary production industries in South Africa. Even though it was found in this study that these organisms readily adhere, attach, colonise and survive on fruit surfaces under artificial inoculation conditions. However,

the likelihood that any of these pathogens will realistically survive on the surface during the cold chain at high enough concentrations to cause illness can be considered highly unlikely. In addition, these organisms have not yet been linked to an outbreak of foodborne illness being traced back to stone fruit production or packing. However, grower continual improvement systems should be implemented to ensure that these pathogens do not get introduced and will not survive the external distribution network.

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Appendix A: Growth dynamics data of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Staphylococcus aureus.*

Table A1.1: Growth dynamics of *Escherichia coli* O157:H7 under varying temperature conditions in a nutrient rich and nutrient poor broth and on a nutrient free tile

Nutrient Conditions	Day	Temperature Conditions			
		Fluctuating	0.5°C	4°C	21°C
Nutrient Rich	0	4.91* **c** [w]	4.87 **ab**	4.86 **b**	4.85 **d**
	1	8.99 **a**	5.26 **a**	5.57 **a**	8.95 **bc**
	2	9.03 **a**	3.62 **c**	5.36 **a**	9.07 **abc**
	3	9.03 **a**	4.77 **b**	5.42 **a**	8.89 **c**
	4	8.79 **b**	4.45 **b**	5.15 **ab**	9.00 **abc**
	5	9.03 **a**	3.82 **c**	5.54 **a**	9.16 **a**
	6	9.03 **a**	3.84 **c**	5.51 **a**	9.10 **ab**
	LSD [x]	0.1761	0.4501	0.4675	0.1864
	***P* value** [y]	<0.0001	<0.0001	0.0587	<0.0001
Nutrient Poor	0	4.97 **d**	4.97 **ab**	4.97 **a**	4.97 **d**
	1	8.33 **c**	3.38 **c**	4.27 **b**	8.13 **c**
	2	8.40 **bc**	4.12 **b**	4.02 **bc**	8.21 **c**
	3	9.36 **a**	3.28 **cd**	3.83 **bc**	8.44 **b**
	4	8.50 **bc**	2.96 **de**	3.46 **c**	8.59 **ab**
	5	8.61 **b**	2.99 **de**	3.90 **bc**	8.63 **a**
	6	8.53 **bc**	2.77 **e**	3.85 **bc**	8.60 **a**
	LSD	0.2328	0.3267	0.6783	0.1532
	***P* value**	<0.0001	<0.0001	0.0112	<0.0001
Nutrient Free	0	ND[z]	0.90 [#] **a**	0.90 **a**	0.90 **a**
	1	ND	0.96 **a**	0.64 **a**	0.96 **a**
	2	ND	0.96 **a**	0.64 **a**	0.00 **a**
	3	ND	0.96 **a**	0.64 **a**	0.32 **a**
	4	ND	0.64 **a**	0.00 **a**	0.32 **a**
	5	ND	0.96 **a**	0.00 **a**	0.32 **a**
	6	ND	0.32 **a**	0.00 **a**	0.00 **a**
	LSD	-	1.2493	1.3128	1.1598
	***P* value**	-	0.8818	0.5661	0.3063

*: all values are represented as cfu/ml; [#]: all values are represented as cfu/cm^2; w: Values followed by the same **bolded letter** means that the two values are not significantly difference according to the Fischer Test (P<0.05); [x]: LSD is the least significant difference within one temperature condition; [y]: *P* value is significant if the value is less than 0.05; [z]: ND means that the specific value is not available because it was not included in the present study.

Table A1.2: Growth dynamics of *Listeria monocytogenes* under varying temperature conditions in a nutrient rich and nutrient poor broth and on a nutrient free tile

Nutrient Conditions	Day	Temperature Conditions			
		Fluctuating	0.5°C	4°C	21°C
Nutrient Rich	**0**	4.98* **c** [w]	4.95 **c**	4.99 **e**	4.93 **e**
	1	9.08 **a**	5.48 **c**	6.07 **d**	8.93 **ab**
	2	9.05 **a**	5.83 **b**	6.61 **cd**	9.19 **a**
	3	8.31 **b**	5.93 **ab**	6.39 **cd**	8.72 **bc**
	4	8.40 **b**	6.15 **ab**	7.36 **bc**	8.24 **d**
	5	8.34 **b**	6.23 **ab**	7.74 **ab**	8.44 **cd**
	6	8.25 **b**	6.33 **a**	8.54 **a**	8.23 **d**
	LSD [x]	0.3575	0.4549	1.0502	0.3492
	***P* value** [y]	<0.0001	0.0002	0.0002	<0.0001
Nutrient Poor	**0**	5.19 **e**	5.19 **b**	5.19 **e**	5.19 **b**
	1	8.48 **b**	5.28 **ab**	5.21 **e**	8.35 **a**
	2	9.11 **a**	5.23 **b**	5.62 **d**	8.29 **a**
	3	9.09 **a**	5.22 **b**	3.99 **f**	8.22 **a**
	4	7.92 **c**	5.35 **ab**	6.41 **cd**	8.38 **a**
	5	6.89 **d**	5.36 **ab**	6.69 **bc**	8.35 **a**
	6	6.90 **d**	5.49 **a**	7.15 **a**	8.28 **a**
	LSD	0.1316	0.2247	0.2127	0.1667
	***P* value**	<0.0001	<0.0001	<0.0001	<0.0001
Nutrient Free	**0**	ND[z]	2.65 **a**	2.65 **a**	2.65 **a**
	1	ND	1.77 **b**	1.93 **b**	2.14 **b**
	2	ND	1.55 **b**	0.96 **c**	2.04 **b**
	3	ND	1.27 **bc**	0.96 **c**	1.58 **c**
	4	ND	0.69 **d**	0.64 **c**	1.05 **d**
	5	ND	0.96 **cd**	0.64 **c**	1.05 **d**
	6	ND	0.96 **cd**	0.00 **d**	0.96 **d**
	LSD	-	0.5386	0.5677	0.2955
	***P* value**	-	<0.0001	<0.0001	<0.0001

*: all values are represented as cfu/ml; [w]: Values followed by the same **bolded letter** means that the two values are not significantly difference according to the Fischer Test (P<0.05); [x]: LSD is the least significant difference within one temperature condition; [y]: *P* value is significant if the value is less than 0.05; [z]: ND means that the specific value is not available because it was not included in the present study.

Table A1.3: Growth dynamics of *Salmonella* Typhimurium under varying temperature conditions in a nutrient rich and nutrient poor broth and on a nutrient free tile

Nutrient Conditions	Day	Temperature Conditions			
		Fluctuating	0.5°C	4°C	21°C
Nutrient Rich	0	5.02* **c** [w]	5.32 **a**	5.00 **c**	5.00 **c**
	1	8.74 **b**	5.26 **a**	5.61 **ab**	7.88 **b**
	2	8.93 **a**	4.88 **a**	5.90 **a**	9.12 **a**
	3	8.74 **b**	5.08 **a**	5.54 **ab**	9.02 **ab**
	4	8.74 **b**	4.93 **a**	5.31 **bc**	8.98 **ab**
	5	9.07 **a**	5.13 **a**	5.69 **ab**	9.09 **ab**
	6	9.04 **a**	5.09 **a**	5.86 **a**	9.16 **a**
	LSD [x]	0.1788	1.0013	0.4284	1.2200
	***P* value** [y]	<0.0001	0.9525	0.0075	<0.0001
Nutrient Poor	0	5.02 **c**	5.02 **a**	5.02 **a**	5.02 **d**
	1	8.12 **b**	4.83 **ab**	4.61 **b**	8.01 **c**
	2	8.09 **b**	4.54 **bc**	3.78 **e**	8.12 **bc**
	3	8.13 **b**	4.39 **cd**	4.48 **bc**	8.25 **ab**
	4	8.23 **ab**	4.11 **de**	4.21 **c**	8.37 **a**
	5	8.31 **a**	4.34 **cd**	4.17 **cd**	8.27 **ab**
	6	8.12 **b**	3.97 **e**	3.84 **de**	8.19 **abc**
	LSD	0.1739	0.3721	0.3721	0.2140
	***P* value**	<0.0001	0.0005	0.0005	<0.0001
Nutrient Free	0	ND[z]	2.40 **a**	2.40 **a**	2.40 **a**
	1	ND	2.23 **a**	1.13 **b**	2.09 **a**
	2	ND	2.47 **a**	1.23 **b**	1.87 **a**
	3	ND	1.11 **b**	0.96 **bc**	1.20 **b**
	4	ND	1.28 **b**	1.05 **bc**	1.05 **b**
	5	ND	0.48 **b**	0.96 **bc**	1.26 **b**
	6	ND	1.28 **b**	0.64 **c**	1.15 **b**
	LSD	-	0.4206	0.4651	0.5776
	***P* value**	-	<0.0001	<0.0001	0.0011

*: all values are represented as cfu/ml; [w]: Values followed by the same **bolded letter** means that the two values are not significantly difference according to the Fischer Test (P<0.05); [x]: LSD is the least significant difference within one temperature condition; [y]: P value is significant if the value is less than 0.05; [z]: ND means that the specific value is not available because it was not included in the present study.

Table A1.4: Growth dynamics of *Staphylococcus aureus* under varying temperature conditions in a nutrient rich and nutrient poor broth and on a nutrient free tile

Nutrient Conditions	Day	Temperature Conditions			
		Fluctuating	0.5°C	4°C	21°C
Nutrient Rich	0	4.96* **d** [w]	4.95 **abc**	4.92 **b**	5.05 **d**
	1	8.52 **a**	5.26 **a**	5.14 **ab**	8.12 **c**
	2	7.73 **ab**	4.98 **ab**	5.93 **ab**	8.26 **c**
	3	7.29 **bc**	4.83 **bc**	5.09 **ab**	8.84 **b**
	4	6.76 **c**	4.71 **bc**	4.47 **b**	9.06 **ab**
	5	7.40 **bc**	4.73 **bc**	4.63 **b**	9.16 **a**
	6	7.05 **bc**	4.66 **c**	4.91 **b**	9.13 **ab**
	LSD [x]	0.9476	0.3093	0.9435	0.3083
	***P* value** [y]	0.0001	0.0153	0.0971	<0.0001
Nutrient Poor	0	5.01 **d**	5.01 **ab**	5.01 **a**	5.01 **d**
	1	7.14 **c**	4.91 **abc**	4.98 **ab**	7.16 **c**
	2	7.02 **c**	4.94 **abc**	4.82 **ab**	8.23 **b**
	3	6.98 **c**	5.03 **ab**	4.86 **ab**	8.59 **a**
	4	8.30 **b**	5.12 **a**	4.92 **ab**	8.75 **a**
	5	9.26 **a**	4.83 **bc**	4.66 **bc**	8.77 **a**
	6	9.38 **a**	4.74 **c**	4.45 **c**	8.74 **a**
	LSD	0.2508	0.2392	0.3360	0.2025
	***P* value**	<0.0001	0.0704	0.0379	<0.0001
Nutrient Free	0	ND [z]	3.22 **a**	3.22 **a**	3.22 **a**
	1	ND	2.86 **bc**	2.52 **b**	2.77 **b**
	2	ND	2.97 **b**	1.54 **c**	2.89 **b**
	3	ND	2.72 **cd**	1.20 **cd**	2.28 **c**
	4	ND	2.75 **cd**	1.08 **d**	2.22 **c**
	5	ND	2.74 **cd**	0.37 **e**	1.59 **d**
	6	ND	2.55 **d**	0.96 **d**	1.28 **e**
	LSD	-	0.2010	0.4420	0.2030
	***P* value**	-	0.0003	<0.0001	<0.0001

*: all values are represented as cfu/ml; [w]: Values followed by the same **bolded letter** means that the two values are not significantly difference according to the Fischer Test (P<0.05); [x]: LSD is the least significant difference within one temperature condition; [y]: P value is significant if the value is less than 0.05; [z]: ND means that the specific value is not available because it was not included in the present study.

Appendix B: Responses to all diagnostic tool questions

	Part 0: Introduction Questions	Peach Farm 1	Peach Farm 2	Plum Farm 1	Plum Farm 2
1	What is the ownership of the farm?	independent	independent	independent	independent
2	Name farm/corporation	Peach Farm 1	Peach Farm 2	Plum Farm 1	Plum Farm 2
3	Location of your farm	Mookgophong, Limpopo	Robertson, Western Cape	Mookgophong, Limpopo	Somerset West, Western Cape
4	Total number of employees in your company? *	50-249	1 to 9	50-249	>249
5	Which product do you cultivate?	Peach	Peach	Plum	Plum
6	What type of cultivation system do you have?	open field	open field	open field	open field
7	Are your products sold as organic production	No	Yes	Yes	No
8	Do you have combined production of cereals or animal by-products?	No	No	No	No
9	Which Quality Assurance (QA) standards/guidelines have been implemented? *	Global GAP	Global GAP and HACCP	GLOBAL GAP	GLOBAL GAP
10	For which QA standards is your company certified? *	Global GAP	SGS Organic certification	GLOBAL GAP	GLOBAL GAP
11	Did the owner/manager of the farm undergo training on food safety/quality management? *	Yes	Yes	Yes	Yes
12	Which specific product is made in this production unit?	Peach	Peach	Plum	Plum
13	Who are the major customers of this specific product group?*	Open markets and Wholesale	Retailers/ wholsesalers	Open markets/ Retailers	Wholesale/ Retail
14	What are the initial materials that you used for this product group?	Small trees	Small trees	Small trees	Small trees
15	What is the packaging/storage concept used for this product group?	punnets/boxes	No packaging, done by retailer	punnets/boxes	punnets/boxes
16	Who are major suppliers of initial materials?*	Nurseries	Nurseries	SAPO	Stemmet Nursery/ Nexus
17	What are the major activities to cultivate this product?	organic fertiliser, organic pesticides, harvesting and packaging	conventional fertiliser, conventional pesticides and harvesting	organic fertiliser, organic pesticides, harvesting and packaging	conventional fertiliser, conventional pesticides, harvesting and packaging
18	What are the major units used for this product group?	field, packing area and cold room	field	field, packing area and cold room	field, packing area and cold room
19	What are the major equipment/machines used for this product group?	pesticide sprayer, tractor for fruit transport and packline	pesticide sprayer and tractor for fruit transport	pesticide sprayer, tractor for fruit transport and packline	pesticide sprayer, tractor for fruit transport and packline

	Part I: Assessment of product factors	Peach Farm 1	Peach Farm 2	Plum Farm 1	Plum Farm 2
	A. Assessment of product characteristics				
	In which situation would you place the:				
A1	initial materials of you RPU in respect to microbiological contamination?	Situation 2	Situation 2	Situation 2	Situation 2
A2	initial materials of you RPU in respect to maximum residue pesticide levels?	Situation 2	Situation 2	Situation 2	Situation 2
A3	initial materials of you RPU in respect to mycotoxin contamination?	Situation 2	Situation 2	Situation 2	Situation 2
A4	final product of you RPU in respect to microbiological contamination?	Situation 2	Situation 2	Situation 2	Situation 2
A5	final product of you RPU in respect to maximum residue pesticide levels?	Situation 3	Situation 1	Situation 1	Situation 3
	B. Assessment of process characteristics				
	In which situation would you place the:				
B6	susceptibility for microbial contamination of the production/cultivation system of your RPU?	Situation 3	Situation 3	Situation 3	Situation 3
B7	climatic conditions in which your RPU operates, in respect to foodborne and chemical contamination?*	Situation 3	Situation 3	Situation 3	Situation 3
B8	water supply of your RPU in respect to foodborne and chemical contamination of your RPU?	Situation 3	Situation 3	Situation 3	Situation 3
Mean product and process assessment		**2.5**	**2.3**	**2.3**	**2.5**
	C. Assessment of organisation characteristics				
	In which situation would you place:				
C9	your company with regards to technological staff?	Situation 3	Situation 3	Situation 3	Situation 3
C10	the variability of workforce composition with respect to your RPU?	Situation 1	Situation 1	Situation 2	Situation 2
C11	operator competences with respect to your RPU?	Situation 3	Situation 3	Situation 3	Situation 3
C12	management commitment in your company?	Situation 3	Situation 3	Situation 2	Situation 3
C13	employee involvement with respect to your RPU?	Situation 3	Situation 3	Situation 3	Situation 3
C14	formalization to support decision-making in your company?	Situation 3	Situation 3	Situation 3	Situation 3
C15	information systems to support food safety (management system) decisions in your company?	Situation 3	Situation 3	Situation 3	Situation 3

D. Assessment of chain characteristics					
	In which situation would you place:				
D16	requirements of stakeholders with respect to your RPU	Situation 2	Situation 2	Situation 2	Situation 2
D17	relationships with respect to the major suppliers of critical materials for you RPU?	Situation 2	Situation 2	Situation 2	Situation 2
D18	your food safety information exchange with the major suppliers of critical materials for you RPU?	Situation 3	Situation 3	Situation 3	Situation 3
D19	the logistical facilities used until the products of your RPU reach the next chain actor?	Situation 1	Situation 3	Situation 1	Situation 1
D20	inspections of food safety authorities in your country in respect to your RPU?	Situation 3	Situation 3	Situation 3	Situation 3
D21	the supply source of initial materials for the cultivation in your farm/company?	Situation 2	Situation 2	Situation 2	Situation 2
D22	the specificity food safety legal framework in your country in respect to your RPU?	Situation 3	Situation 3	Situation 3	Situation 3
D23	the hygienic design of equipment and facilities relevant for your RPU?	Situation 3	Situation 3	Situation 3	Situation 3
Mean organisation and chain		**2.5**	**2.7**	**2.5**	**2.6**
Part II: Assessment of core control activities					
E. Assessment of preventive measure design					
	In which situation would you place:				
E24	the hygienic design of equipment and facilities relevant to your RPU?	Situation 2	Situation 1	Situation 2	Situation 2
E25	your maintenance and calibration program relevant to you RPU?	Situation 2	Situation 1	Situation 2	Situation 2
E26	the storage facilities relevant to your RPU?	Situation 4	Situation 3	Situation 4	Situation 4
E27	the sanitation program(s) relevant to your RPU?	Situation 2	Situation 1	Situation 2	Situation 2
E28	the personal hygiene requirements relevant to your RPU?	Situation 3	Situation 3	Situation 3	Situation 3
E29	the incoming material control relevant to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
E30	your packaging equipment relevant to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
E31	the supplier control relevant to your RPU?	Situation 3	Situation 2	Situation 3	Situation 3
E32	the organic fertiliser program relevant to your RPU?	Situation 3	Situation 3	Situation 3	Situation 3
E33	the pesticide program relevant to your RPU?	Situation 3	Situation 1	Situation 1	Situation 3
E34	the water control relevant to your RPU?	Situation 2	Situation 2	Situation 2	Situation 2
E35	the irrigation method relevant to your RPU?	Situation 3	Situation 3	Situation 3	Situation 3

F. Assessment of intervention process design					
	In which situation would you place:				
F36	your physical intervention relevant to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
G. Assessment of monitoring system design					
	In which situation would you place:				
G37	analytical methods to assess pathogens with respect to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
G38	analytical methods to assess the pesticides maximum residue level with respect to your RPU?*	Situation 3	Situation 1	Situation 1	Situation 4
G39	sampling plan for microbial assessment with respect to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
G40	sampling plans for maximum residue level of pesticide assessment with respect to your RPU?*	Situation 2	Situation 1	Situation 1	Situation 3
G41	corrective actions with respect to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
Mean control activities design		**2.1**	**1.6**	**1.8**	**2.2**
H. Assessment of operation of preventative measures, intervention processes and monitoring systems					
	In which situation would you place the actual:				
H42	availability of procedures in your RPU?	Situation 2	Situation 2	Situation 2	Situation 2
H43	availability of compliance to procedures in your RPU?	Situation 2	Situation 2	Situation 2	Situation 2
H44	hygiene performance of equipment and facilities with respect to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
H45	storage/cooling capacity in your RPU?	Situation 4	Situation 3	Situation 4	Situation 4
H46	process capability of partial physical intervention with respect to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
H47	process capability of packaging processes with respect to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
H48	performance of analytical equipment relevant to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
Mean control activities operation		**1.7**	**1.6**	**1.7**	**1.7**
Part III: Assessment of core assurance activities					
I. Assessment setting of system requirements					
	In which situation would you place the:				
I49	translation of stakeholder requirements into own HSMS requirements related to your RPU?	Situation 2	Situation 2	Situation 2	Situation 2
I50	systematic use of feedback information to modify HSMS related to your RPU?	Situation 2	Situation 2	Situation 2	Situation 2

J. Assessment of validation activities					
	In which situation would you place validation of:				
J51	preventative measures with respect to your RPU?	Situation 2	Situation 2	Situation 2	Situation 2
J52	intervention processes (partial physical intervention) with respect to your RPU?	Situation 1	Situation 1	Situation 1	Situation 1
K. Assessment of verification activities					
	In which situation would you place verification of:				
K53	people related performance with respect to your RPU?	Situation 2	Situation 2	Situation 2	Situation 2
K54	equipment and methods related performance with respect to your RPU?	Situation 2	Situation 1	Situation 2	Situation 2
L. Assessment of documentation and record-keeping					
	In which situation would you place:				
L55	documentation with respect to your company?	Situation 3	Situation 3	Situation 3	Situation 3
L56	record keeping with respect to your company?	Situation 3	Situation 3	Situation 3	Situation 3
Mean assurance activities		**2.1**	**2.0**	**2.1**	**2.1**
Part IV: Assessment of core assurance activities					
M. EXTERNAL Food safety performance indicators					
	How would you:				
M57	typify your Food Safety Management System evaluation?	Situation 3	Situation 1	Situation 3	Situation 3
M58	indicate seriousness of remarks of the HSMS evaluation?*	Situation 4	Situation 1	Situation 4	Situation 4
M59	typify the hygiene related and microbiological food safety complaints of customers?*	Situation 4	Situation 1	Situation 4	Situation 1
M60	typify the chemical safety complaints of customers?*	Situation 4	Situation 1	Situation 4	Situation 1
M61	typify the (visual) quality complaints by your customers?	Situation 4	Situation 4	Situation 4	Situation 4
N. INTERNAL Food safety performance indicators					
N62	How would you typify your product sampling to confirm microbiological performance?	Situation 1	Situation 1	Situation 1	Situation 1
N63	Which judgement criteria are used to interpret microbiological results?	Situation 1	Situation 1	Situation 1	Situation 1
N64	How would you typify your product sampling to determine the pesticides residue concentration?	Situation 2	Situation 1	Situation 1	Situation 3
N65	Which judgement criteria are used to interpret pesticide residue results?	Situation 2	Situation 1	Situation 1	Situation 3
N66	How would you typify your non-conformities?	Situation 3	Situation 3	Situation 3	Situation 3
Mean food safety output		**2.8**	**1.3**	**2.6**	**2.4**

Appendix C: Publications and Presentations

The following publications and presentations stemmed from this research:

Publications in Peer Reviewed Journals:

Collignon, S. and Korsten, L. 2010. Attachment and colonization of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* to stone fruit surfaces and survival through a simulated commercial export chain. *Journal of Food Protection* **73:** 1247-1256.

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Collignon, S. and Korsten, L. 2008. Packhouse hygiene, SASPA Farmers Day, Naboomspruit, South Africa, 5 September 2008

Collignon, S. and Korsten, L. 2008. Attachment potential of Important Foodborne Pathogens to Plums, Microscopy Society of Southern Africa, Gaborone, Botswana, 7-11 July 2008

Proceedings:

Collignon, S. and Korsten, L. 2008. Attachment potential of Important Foodborne Pathogens to Plums, Microscopy Society of Southern Africa Proceedings, 38: 47. Gaberone, Botswana.

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Attachment and Colonization by *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium, and *Staphylococcus aureus* on Stone Fruit Surfaces and Survival through a Simulated Commercial Export Chain

STACEY COLLIGNON AND LISE KORSTEN*

Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa

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ABSTRACT

The ability of the foodborne pathogens *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium, and *Staphylococcus aureus* to attach, colonize, and survive on stone fruit surfaces was investigated. Fifty microliters of bacterial suspension was spot inoculated onto the sterile intact fructoplane of whole peaches and plums. Minimum time required for initial adhesion and attachment was recorded for different surface contact times. Surface colonization patterns of the four pathogens and survival under simulated commercial export conditions also were evaluated. *L. monocytogenes* and *Salmonella* Typhimurium attached immediately to stone fruit surfaces. *E. coli* O157:H7 and *S. aureus* were visibly attached after 30 s and 1 h, respectively, of direct exposure. Holding freshly harvested stone fruit at 0.5°C to simulate cold storage conditions significantly lowered the titer of *E. coli* O157:H7 on plums and the titers of *L. monocytogenes* and *Salmonella* Typhimurium on stone fruit. *E. coli* O157:H7 and *L. monocytogenes* at a low inoculum level and *S. aureus* and *Salmonella* Typhimurium at high and low levels did not survive the simulated export chain conditions at titers that exceeded the minimum infectious dose. However, *E. coli* O157:H7 and *L. monocytogenes* were able to survive on stone fruit surfaces when inoculated at an artificially high level. In this case, the final titer at the end of the supply chain was higher than the infectious dose. In this laboratory experiment, *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium, and *S. aureus* at potential natural contamination levels were unable to survive simulated export conditions.

The number of human disease outbreaks associated with foodborne pathogens has increased globally, and this change has been linked to increased consumption of contaminated fresh produce *(51)*. The increase in the reported number of outbreaks can be attributed to a various factors, including the shifting focus toward healthier lifestyles and diets in more developed countries. Because of the demand for year-round availability of fresh fruit and vegetables and more exotic produce, these products often are procured from less developed countries with less effectively regulated food control systems. These global procurement patterns have resulted in more extensive supply chains, ultimately involving more complex distribution networks and longer road and sea transit times. More complex distribution systems in turn require more handling. *Staphylococcus aureus* is transmitted through food handlers and therefore is an important consideration in any food safety implementation system. *S. aureus* also has been linked to foodborne outbreaks throughout the world. Foodborne illnesses associated with the consumption of contaminated cantaloupe, cut fruit, strawberries, raspberries, tomatoes, spinach, lettuce, and various other types of fresh produce have been well documented *(8–13, 24, 30, 35, 46)*. Some of the more frequently reported foodborne pathogens associated with fresh produce are *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella (14)*. Both *E. coli* and *Salmonella* have been detected on the surfaces of stone fruits *(1)*.

Microbial contamination of fresh produce can occur within the pre- and postharvest environments. The exposure of fresh produce to contaminated water, handlers, or contact surfaces *(5, 28, 44)* increases the likelihood that foodborne pathogens can successfully attach to the fructoplane. Contamination should therefore be avoided by implementing pre- and postharvest production and distribution practices that prevent contamination. Fresh produce that is traded through extensive supply chains also are exposed to several possible contamination points after leaving the farm. Contamination could therefore occur at any point from the farm and packing house up to the point of handling and consumption within the importing country *(31)*.

* Author for correspondence. Tel: +27 12 420 3295; Fax: +27 12 420 4588; E-mail: lise.korsten@up.ac.za.

Environmental conditions during transit of the fresh product are important because they can either support microbial growth and survival or contribute to the death of these organisms, thereby either increasing or reducing the food safety risk. Stringent controls at the point of production and dispatch are required to ensure that foodborne pathogens are not introduced into the food chain. When contamination is possible, intervention strategies should be implemented to ensure that the organism cannot survive or proliferate up to the point of consumption. Effective cold chain management systems can prevent the proliferation of foodborne pathogens on fresh produce surfaces. *E. coli, L. monocytogenes, Salmonella,* and *S. aureus* are able to survive refrigeration temperatures, and *L. monocytogenes* is able to grow at the temperatures *(2, 16, 19, 54).*

Adherence, attachment, colonization, and survival of foodborne pathogens on raw fresh produce is a critical element in the fruit contamination cycle *(32).* Understanding the stages of the organisms' contamination cycle will allow the establishment of better prevention strategies within the pre- and postharvest environment. The plant surface and the bacterial cell both have a negative charge *(53).* Adhesion is a physiochemical process that occurs when a bacterial cell is able to overcome the repulsive forces between the cell and the plant surface *(22, 53).* Adhered cells are able to detach if repulsive forces become greater than attractive forces *(22).* Adhered cells become attached by means of exopolysaccharides. Once attached, cells are able to replicate to form microcolonies *(37),* which promote survival. For foodborne pathogens to survive on the plant's phyllo- or fructoplane, the bacteria must transport and utilize available nutrients *(43).* Fett *(20)* suggested that human pathogens may become incorporated into phylloplane biofilms. Microorganims found on the phylloplane often are found in biofilms, which buffer environmental fluctuations *(38, 40, 41).* These biofilms often are associated with sources of nutrients such as the leaf vein and trichomes *(40).*

The aim of this study was to acquire a better understanding of the potential of *E. coli* O157:H7, *L. monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium, and *S. aureus* to adhere, attach, colonize, and survive on stone fruit. Time and temperature exposure conditions that simulated harvesting, packing, transport, cold storage, and export conditions used to retain fruit quality, control decay, and extend shelf life were used to determine the likelihood of foodborne pathogens survival on stone fruit surfaces.

MATERIALS AND METHODS

Cultures. American Type Culture Collection (ATCC) cultures of *E. coli* O157:H7 (ATCC 35150), *L. monocytogenes* (ATCC 19115), *S. enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028), and *S. aureus* (ATCC 12600) were used as reference cultures in this study. All cultures were maintained lyophilized and stored at −70°C, and subcultures were grown on standard 1 medium (Merck, Johannesburg, South Africa) prepared 24 h before use. Cultures were used to inoculate five replicates of 100 ml of tryptone soy broth (Merck) for each pathogen and were subsequently incubated at 37°C for 18 h to achieve 8 log CFU/ml. Cultures were centrifuged at 2,200 × *g,* washed twice with sterile distilled water, and then resuspended in 1% (wt/vol) peptone buffered water (Merck). Cultures were then serially diluted to obtain a high inoculum level of 7 log CFU/ml and a low inoculum level of 5 log CFU/ml. Levels were confirmed by serial dilution and subsequent plating in duplicate.

Fruit. Peaches (*Prunus persica* cv. Excellence) and plums (*Prunus domestica* cv. Flavour King) were aseptically hand harvested at optimum maturity from two commercial farms in the North West Province and Limpopo Province, respectively, of South Africa. The full experiment was repeated on two separate occasions. Fruits of a uniform size and weight and without pests, disease, and damage were used in this study. Harvested fruits were bagged in paper bags and transported to the laboratory in cooler boxes and stored at 4°C overnight (approximately 12 to 15 h). Collected fruits were divided into three sets. Set 1 was used for scanning electron microscopy (SEM) analysis and consisted of 22 peaches (7 for each pathogen and 1 negative control) and 37 plums (9 for each pathogen and 1 negative control). Set 2 was used to quantify the pathogen titer following high-level inoculation and consisted of 50 peaches (5 replicates for 9 time intervals selected plus 5 negative controls) and 62 plums (5 replicates for 11 time intervals and 7 negative controls). Set 3 was used to quantify the pathogen titer following low-level inoculation and consisted of 30 peaches (5 replicates for 5 day intervals and 5 negative controls) and 42 plums (5 replicates for 7 day intervals and 7 negative controls). Set 1 fruits for SEM studies were surface sterilized with a 30-s dip treatment in 70% ethanol *(49)* followed by air drying. Fruits from sets 2 and 3 were washed with 0.05% (vol/vol) sodium hypochlorite for 30 s, rinsed twice with sterile distilled water, and allowed to air dry.

Spot inoculation. Spot inoculation for SEM studies was done on a surface area (5 by 5 mm) that was marked with a felt pen; 50 μl of prepared culture was used per pathogen for short time intervals (0, 30, and 60 s and 1 h) to determine attachment and for longer time intervals for peaches (1, 14, 20, and 21 days) and plums (1, 6, 13, 18, 25, and 26 days) using the high-level inoculum (7 log CFU/ml) (Fig. 1). For the attachment studies, the culture was put directly onto the fruit at room temperature and aspirated at the respective time intervals. The inoculated fruit surface area was subsequently rinsed with 100 μl of sterile distilled water on the inoculated section. The rinsate was then aspirated and discarded, and the rinsing process was repeated. The inoculated blocks were then aseptically excised and immediately processed for SEM (set 1). Spot inoculation with using 50 μl of culture for determination of pathogen titers was carried out on five set 2 (high-level inoculum) and five set 3 (low-level inoculum) fruit per short time interval (0, 30, and 60 s and 1 and 2 h) and long time intervals (Fig. 1). Spot inoculation was carried out ensuring that cultures were not mixed. After spot inoculation, the final level of each culture on the fruit was confirmed to be 5 (high) and 3 (low) log CFU per fruit with serial dilutions as described before. The titers of viable *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium, and *S. aureus* remaining on these fruits at the various time intervals was determined. Once inoculated, fruit were divided according to replicates (five each for pathogen titer determination and four each for SEM) and were distributed equally into five containers in five areas of the incubation space to allow for temperature variation within the incubation chamber.

Methodology for quantification of microorganisms. Inoculated set 2 and set 3 fruits (five replicates) were used to quantify

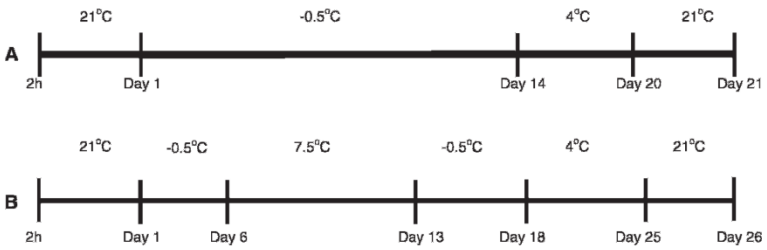

FIGURE 1. *Time regimen for the ship freight export simulation experiment for peaches (A) and plums (B).*

pathogen titers after the various short and long time intervals. Fruits were removed from the incubation cold storage area at the different time intervals and washed to determine the bacterial titer present. Fruits were washed in 500 ml of 0.25× Ringer's solution amended with 0.02% Tween 80 (Sigma, Johannesburg, South Africa) in the Ultrasonic Bath (Labotec, Johannesburg, South Africa) for 30 s. The Ringer's solution was then filtered through a 0.45-nm-pore-size nitrocellulose membrane, and the membrane was used for serial dilution and plating in duplicate onto selective agar specific for the four pathogens: Baird-Parker medium for *S. aureus*, Oxford *Listeria* selective agar for *L. monocytogenes,* Levine eosin–methyl blue agar for *E. coli* O157:H7, and xylose lysine deoxycholate agar for *Salmonella* Typhimurium (all supplied by Merck). Volume displaced (vd) for each fruit was recorded and converted to area (square centimeters) with the following equation *(17)*:

$$A = 4.84\left[(\mathrm{vd})^{1/3}\right]^2$$

Counts were converted to CFU per square centimeter and transformed to $\log(x + 1)$ CFU/cm^2.

SEM evaluation. Marked and inoculated sections of set 1 fruits were used for SEM evaluation. The uninoculated fruits served as negative controls. Excised sections were stored in 1 ml of fixing solution, which consisted of 1 ml of 25% gluteraldehyde in 0.075 M phosphate buffer mixed according to the method of Coetzee and Van der Merwe *(15)* with a modification of 25% formaldehyde. Samples were stored for a maximum of 1 month. Samples were rinsed three times in 0.075 M phosphate buffer for 15 min each, followed by successive 15-min dehydration rinses in 50, 70, and 90% ethanol and finally three rinses in 100% ethanol. Samples were critical point dried in a Bio-Rad dryer (Bio-Rad, Polaron Equipment, Watford, Hertfordshire, UK) under liquid carbon dioxide. After drying, samples were mounted with nonconductive tape and coated for 2.5 min with 10 mÅ of gold-palladium (Polaron Equipment) and examined under a scanning electron microscope (JSM-840, JEOL Ltd., Tokyo, Japan) operating at 5 or 8 kV. Negative controls were examined first to become familiar with the fructoplane. The next samples examined were those inoculated with the higher titer and allowed to grow for longer time intervals (21 or 26 days) to determine the orientation and size of the bacterial pathogens. The rest of the samples were then examined systematically through all samples from the longest exposure times (highest titers) to the shortest exposure times (lowest titers). Cells were counted on 15 randomly selected areas per sample at ×3,000 magnification. The following equation was generated to calculate the number of pathogen cells per square centimeter of fruit:

$$\text{cells per cm}^2 = (c)(a/y)^{-1}$$

where c is the number of pathogen cells counted, a is the area of the SEM viewing section, and y is the average area per fruit (111.15 cm^2) as determined in the present study (described previously).

Observations were made for 15 areas per sample on each stub to determine adhesion, attachment, replication, colonization, and survival of the organisms on the fruit surfaces. Observations were subsequently transformed into a percentage of observations per sample viewed (i.e., the frequency). Thus, the attachment frequency is the percentage of observed attachment, and the replication frequency is the percentage of observed multiplication.

Statistical analysis. All experiments were repeated. Results obtained for each replicate were analyzed together, for a total of 10 replicates. Statistical analysis was performed on log CFU per square centimeter and log cells per square centimeter. Data were analyzed using SAS 9.2 for Windows (SAS Institute Inc., Cary, NC). A one-way analysis of variance was used to determine the difference in pathogen titers on fruit surfaces. Means were analyzed using the least significant difference (using the Fisher test) at a 5% level of significance.

RESULTS

Surface characteristics. The uninoculated control samples examined revealed the fruit surface characteristics, i.e., trichomes, lenticels, wax structures, and epidermal corrugation. Examination of 420 areas revealed that the peach surfaces were extensively covered with trichomes (Fig. 2A). Because of this high incidence of trichomes on this specific cultivar, other surface characteristics were difficult to discern. The peach surface did not appear to have many lenticels. Examination of 540 plum areas revealed a smooth surface without much corrugation and with lenticels and smooth wax plates (Fig. 3A). No microorganisms were found by SEM examination of the control fructoplane, indicating that the ethanol surface sterilization process was successful due to toxic activity on resident microflora *(49)*. The morphological characteristics of the bacterial pathogens on the fruit surfaces were observed for fruits from the high-level inoculation group. Morphological characteristics were consistent for all bacterial pathogens viewed at the simulated longest exposure time period.

All four pathogens preferentially attached to the trichomes on peaches (Fig. 2) and were evenly distributed over the trichome. On plums, all pathogens were able to attach to the smooth surface of the fruit but preferentially attached to areas near lenticels.

Initial adhesion. Initial adhesion was defined as the first time interval for which microbial counts (CFU per square centimeter) for the high- and low-level inoculum groups were obtained and when cells (not attached with

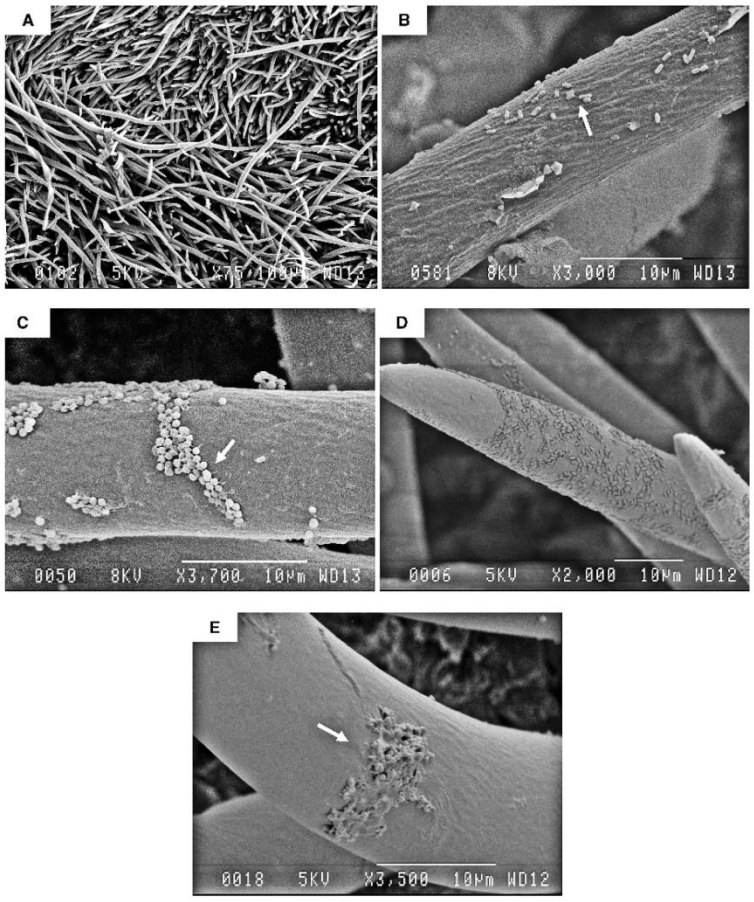

FIGURE 2. *Electron micrographs of foodborne bacterial pathogens on peaches. (A) Trichomes on the surface of peaches (×75 magnification). Arrows indicate the point of attachment with exopolysaccharides by (B)* Listeria monocytogenes *(×3,000 magnification) and (C)* Staphylococcus aureus *(×3,700 magnification). (D) Microcolony formation of* L. monocytogenes *after 1 h on a peach trichome (×2,000 magnification). (E) Microcolony formation of* L. monocytogenes *is indicated with arrows (×3,500 magnification).*

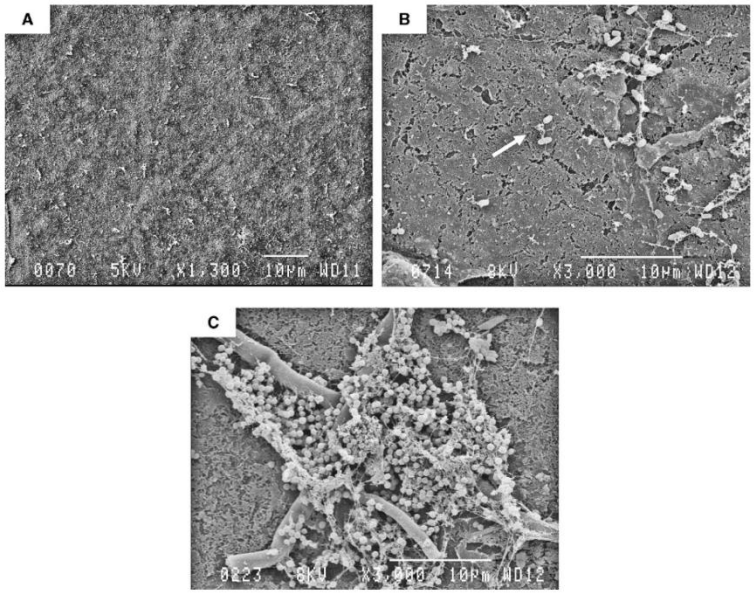

FIGURE 3. *Electron micrographs of foodborne bacterial pathogens on plums. (A) Smooth surface of plums (×1,300 magnification). (B) Arrow indicates the point of attachment with exopolysaccharides of* Salmonella enterica *subsp.* enterica *serovar Typhimurium (×3,000 magnification). (C) Extensive* Staphylococcus aureus *microcolony formation (×3,000 magnification) after 26 days of the export cold chain.*

TABLE 1. *Shortest time required for adhesion and attachment of the high-level inoculum of* Escherichia coli *O157:H7,* Listeria monocytogenes, Salmonella *Typhimurium, and* Staphylococcus aureus *to stone fruits*

Pathogen	Adhesion time		Attachment time	
	Peaches	Plums	Peaches	Plums
Escherichia coli O157:H7	30 s	0 s	60 s	60 s
Listeria monocytogenes	60 s	0 s	1 h	30 s
Salmonella Typhimurium	0 s	0 s	1 h	30 s
Staphylococcus aureus	0 s	0 s	21 days	1 h

polysaccharides) were first observed on the fruit by SEM. Adherence of *Salmonella* Typhimurium and *S. aureus* occurred immediately after inoculation on both fruits, whereas on peaches *E. coli* O157:H7 adhered within 30 s and *L. monocytogenes* adhered within 60 s. For both of these pathogens adherence to plums was immediately (Table 1). High numbers of *S. aureus* cells were observed by SEM immediately after inoculation and at 2 h postinoculation, and colony counts were high on both peaches and plums, indicating the ability of *S. aureus* to adhere to stone fruit when inoculated at both high and low levels (Tables 2 and 3). All four test organisms were able to adhere to stone fruit when inoculated at the low level but had different viable counts initially (Tables 2 and 3).

Attachment. Attachment of bacteria was defined in this study as the organism's ability to produce exopolysaccharide structures. The first attachment of *E. coli* O157:H7 due to exopolysaccharides on peaches and plums was observed after 60 s of exposure (Tables 1 through 3). The first attachment of *L. monocytogenes* and *Salmonella* Typhimurium was observed on plums 30 s postinoculation (Tables 1 and 3 and Fig. 3B) and on peaches 1 h postinoculation (Tables 1 and 2 and Fig. 2B). *S. aureus* was able to visibly attach to the plum surface via attachment structures at 1 h postinoculation (Tables 1 and 3) and to peaches at 21 days postinoculation (Tables 1 and 3 and Fig. 2C). Organisms were able to attach more effectively to the plum than to the peach surface (Tables 1 through 3).

Colonization. For the purpose of this study, colonization was defined as reproduction of the organism on the fruit surface and the formation of extensive attachment structures. Over time, the amount and frequency of exopolysaccharide production by *E. coli, L. monocytogenes, Salmonella* Typhimurium, and *S. aureus* increased on both types of fruit (Tables 2 and 3). Most notable colonization with attachment structures was observed for *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium, and *S. aureus* on the peach surface toward the end of the fruit export chain (Tables 2 and 3). After 21 days, *L. monocytogenes* and *S. aureus* formed microcolonies (Figs. 2E, 3B, and 3C). No *E. coli* O157:H7 replication was observed on the peach surface between 30 s and 1 h (Table 2), and there was no significant difference in the number of cells or CFU per square centimeter recovered from the peaches (Table 2). However, on plums *E. coli* O157:H7 replication was observed by SEM at 60 s and 1 h postinoculation (Table 3), and a significant increase in CFU per square centimeter was noted from 30 s to 1 h (Table 3). *L. monocytogenes* occurred more prominently on peaches at 1 h postinoculation than at 30 and 60 s (Table 2), and the observed replication frequency was 20% at the 1-h time interval (Table 2). A significant increase in the *L. monocytogenes* counts (cells and CFU per square centimeter) was observed on plums from 30 s to 1 h and from 30 s to 1 day, respectively (Table 3), and replication was observed 1 h postinoculation (Table 3 and Fig. 2D). *Salmonella* Typhimurium significantly increased on peaches and plums from 30 s to 1 day postinoculation (Tables 2 and 3), with the highest observed replication occurring 1 h postinoculation on both fruits (Tables 2 and 3). No significant difference was found in *S. aureus* cell counts on peaches, even though a significant increase was observed in CFU per square centimeter (Table 2). No *S. aureus* replication was found on peaches or plums during these time intervals (Tables 2 and 3). An overall increase in *S. aureus* per square centimeter was observed from 30 s to 1 day postinoculation on both fruits (Tables 2 and 3).

Pathogen survival. Survival was defined in this study as the ability of the organism to survive on the fructoplane throughout the simulated export chain. The 0.5°C incubation period of 1 to 13 days had no significant effect on *E. coli* O157:H7 numbers on the peach surface. However, a significant increase was observed following 4°C storage for 13 to 20 days, with no significant difference between 20 and 21 days (Table 2) even though replication was observed (Table 2). No significant difference was seen in *E. coli* O157:H7 titers when inoculated at the low level on plums (Table 3). However, a significant difference was observed in *E. coli* O157:H7 titer on plum surfaces inoculated at the high level and exposed to 0.5°C for 1 to 6 days (Table 3). The number of cells per square centimeter observed on the plum surface by SEM increased slightly but not significantly, as confirmed by consistent replication of *E. coli* O157:H7 from 30 s to 25 days (Table 3). A significant decrease in *L. monocytogenes* and *Salmonella* Typhimurium was observed between 1 and 6 days (at 0.5°C) on both peaches and plums, with no significant difference observed later in the simulated cold chain, reflecting equilibrium reached where the replication and extinction rates were similar (Tables 2 and 3). *S. aureus* titers did not reflect a significant decrease on peaches (Table 2), and no replication was observed by SEM (Table 2). On plums, there was an overall decrease in *S. aureus* numbers with the most significant decrease occurring 13 days after inoculation (Table 3).

DISCUSSION

The attachment of foodborne pathogens to surfaces of various types of fresh produce has not been widely studied

TABLE 2. *Scanning electron microscopy examination results and total foodborne pathogen counts in the simulated peach export chain experiment*

Pathogen	Time	Attachment frequency (%)[a]	Replication frequency (%)[b]	Log counts[c]		
				SEM (x + 1 cells/cm^2)	High (x + 1 CFU/cm^2)	Low (x + 1 CFU/cm^2)
E. coli O157:H7	0 s[d]	0.00	0.00	0.00 D	1.81 BC	NI[e]
	30 s	0.00	0.00	0.49 CD	1.66 BC	NI
	60 s	6.67	0.00	1.58 CD	2.03 ABC	NI
	1 h	6.67	0.00	1.47 CD	2.47 AB	NI
	2 h[f]	NI	NI	NI	3.21 A	0.51 AB
	1 day	NI	NI	NI	2.02 ABC	0.00 B
	13 days	0.00	0.00	2.18 BC	0.96 C	0.00 B
	20 days	6.67	6.67	3.99 B	2.23 AB	1.03 A
	21 days	33.33	6.67	5.91 A	1.86 BC	0.07 B
L. monocytogenes	0 s[d]	0.00	0.00	0.00 CD	2.41 BC	NI
	30 s	0.00	0.00	0.00 C	2.11 BC	NI
	60 s	0.00	0.00	4.10 B	2.49 BC	NI
	1 h	13.33	20.00	6.11 B	3.82 A	NI
	2 h[f]	NI	NI	NI	3.74 A	2.30 A
	1 day	NI	NI	NI	2.34 BC	0.58 BC
	13 days	0.00	20.00	6.40 A	1.61 C	0.07 C
	20 days	26.67	33.33	6.66 A	1.88 BC	0.81 B
	21 days	6.67	20.00	6.39 A	3.09 AB	0.99 B
Salmonella Typhimurium	0 s[d]	0.00	0.00	0.49 C	0.54 AB	NI
	30 s	0.00	0.00	0.52 C	0.52 AB	NI
	60 s	0.00	6.67	4.10 B	0.87 AB	NI
	1 h	20.00	13.33	6.11 A	1.53 A	NI
	2 h[f]	NI	NI	NI	1.43 A	0.31 A
	1 day	NI	NI	NI	0.88 AB	0.00 A
	13 days	0.00	0.00	6.40 A	0.23 B	0.01 A
	20 days	0.00	6.67	6.71 A	0.75 B	0.04 A
	21 days	40.00	26.67	6.26 A	0.38 B	0.23 A
S. aureus	0 s[d]	0.00	0.00	1.60 C	2.77 CD	NI
	30 s	0.00	0.00	4.58 B	2.61 CD	NI
	60 s	0.00	0.00	5.61 AB	2.43 D	NI
	1 h	0.00	0.00	5.98 AB	3.48 BC	NI
	2 h[f]	NI	NI	NI	4.55 A	2.29 A
	1 day	NI	NI	NI	4.19 AB	1.74 A
	13 days	0.00	0.00	6.77 A	4.47 AB	1.61 AB
	20 days	0.00	0.00	1.19 C	3.55 BC	2.21 A
	21 days	20.00	0.00	6.70 A	3.18 CD	0.78 B

[a] Percentage of observed attachment.
[b] Percentage of observed multiplication.
[c] Letters indicate the least significant difference according to the Fisher test ($P < 0.05$).
[d] After inoculation, culture was immediately aspirated.
[e] NI, not included. Value was not included because it was not an important consideration within this study.
[f] After initial inoculation and drying for the export chain experiment.

but is of great significance to the food industry *(47)*. Most studies focus on processed fruit in the postharvest environment and not on preharvest contamination or on fruits within the supply chain *(6)*.

Colonization and survival on fresh produce is dependent on the ability of foodborne pathogens to adapt to ecological niches outside the host. Traditionally, foodborne pathogens were of little importance on fresh produce, but recent reports of survival and colonization of *E. coli* and *S. enterica* have provided evidence that contamination with these pathogens might represent a food safety risk *(6)*. Pathogens tested in this study survived on peach and plum surfaces. The promotion of growth due to postharvest treatments allows for the pathogen population to be sustained to levels above the minimum infectious dose.

In this study, *S. aureus* adhered to stone fruit surfaces within 30 s. *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* Typhimurium required 1 min to attach to peach surfaces, and *L. monocytogenes* and *Salmonella* Typhimurium required 1 h to adhere to plum surfaces. In general, adhesion occurred more rapidly on peach than on plum surfaces. Solomon and Matthews *(47)* found that heat-killed

TABLE 3. *Scanning electron microscopy examination results and total foodborne pathogen counts in the simulated plum export chain experiment*

Pathogen	Time	Attachment frequency (%)[a]	Replication frequency (%)[b]	Log counts[c] SEM (x + 1 cells/cm^2)	High (x + 1 CFU/cm^2)	Low (x + 1 CFU/cm^2)
E. coli O157:H7	0 s[d]	0.00	6.67	0.33 DE	0.57 CD	NI[e]
	30 s	0.00	0.00	0.57 DE	0.65 CD	NI
	60 s	33.30	33.33	2.75 B	2.08 AB	NI
	1 h	46.67	20.00	4.22 A	2.90 A	NI
	2 h[f]	NI	NI	NI	2.68 AB	0.74 A
	1 day	0.00	6.67	NI	2.01 B	0.12 A
	6 days	NI	NI	0.34 DE	0.43 CD	0.18 A
	13 days	33.33	6.67	1.29 CD	0.05 CD	0.07 A
	18 days	6.67	13.33	2.33 BC	0.10 CD	0.16 A
	25 days	80.00	26.67	3.05 B	0.92 C	0.57 A
	27 days	0.00	0.00	0.00 E	0.03 D	0.13 A
L. monocytogenes	0 s[d]	0.00	0.00	1.29 BC	0.71 FG	NI
	30 s	6.67	0.00	0.77 C	1.62 DE	NI
	60 s	0.00	0.00	2.36 B	2.45 CD	NI
	1 h	13.33	66.67	3.93 A	3.62 AB	NI
	2 h[f]	NI	NI	NI	3.04 BC	0.60 B
	1 day	NI	NI	NI	4.19 A	1.70 A
	6 days	20.00	33.33	3.90 A	1.41 EF	0.13 C
	13 days	60.00	20.00	4.83 A	1.07 EF	0.12 C
	18 days	93.33	13.33	4.75 A	1.05 EF	0.10 C
	25 days	53.33	6.67	4.15 A	0.09 G	0.02 C
	27 days	26.67	6.67	1.69 BC	0.69 FG	0.03 C
Salmonella Typhimurium	0 s[d]	0.00	0.00	0.94 C	2.08 A	NI
	30 s	20.00	26.67	3.20 AB	1.96 A	NI
	60 s	0.00	0.00	2.19 BC	2.10 A	NI
	1 h	33.33	40.00	3.08 AB	2.36 A	NI
	2 h[f]	NI	NI	NI	2.40 A	0.49 A
	1 day	NI	NI	NI	2.81 A	0.22 A
	6 days	20.00	0.00	2.33 ABC	0.76 B	0.04 A
	13 days	0.00	0.00	2.58 AB	0.93 B	0.06 A
	18 days	40.00	13.33	3.70 A	2.08 B	0.00 A
	25 days	6.67	0.00	2.19 BC	0.61 B	0.00 A
	27 days	33.33	20.00	2.41 AB	0.11 B	0.11 A
S. aureus	0 s[d]	0.00	0.00	3.50 DEF	1.85 D	NI
	30 s	0.00	0.00	2.98 F	2.20 D	NI
	60 s	0.00	0.00	3.27 EF	2.19 D	NI
	1 h	13.33	0.00	4.69 BC	3.64 BC	NI
	2 h[f]	NI	NI	NI	4.54 AB	2.51 A
	1 day	NI	NI	NI	4.97 A	1.93 AB
	6 days	100.00	0.00	5.67 A	4.44 ABC	1.63 BC
	13 days	60.00	13.33	4.89 AB	3.57 C	0.75 D
	18 days	26.67	0.00	3.92 CDE	1.94 D	1.23 BCD
	25 days	26.67	0.00	4.27 BCD	2.49 D	1.07 CD
	27 days	66.67	6.67	3.64 DEF	1.99 D	0.62 D

[a] Percentage of observed attachment.
[b] Percentage of observed multiplication.
[c] Letters indicate the least significant difference according to the Fisher test ($P < 0.05$).
[d] After inoculation, culture was immediately aspirated.
[e] NI, not included. Value was not included because it was not an important consideration within this study.
[f] After initial inoculation and drying for the export chain experiment.

bacteria could adhere to lettuce leaves, demonstrating that no physiological activity was required for adhesion.

Adhesion and attachment are essential for colonization and survival. Attachment is a mechanism that ensures that the bacterial cells are not dislodged from the surface once colonization is triggered. Following the initial interaction (adhesion) between the bacteria and the plant, attachment follows if the bacteria are able to utilize the surface nutrients. Even though initial adhesion occurred more quickly on peaches, attachment by means of observed

attachment structures was observed earlier on plums than on peaches. In this study, attachment occurred as early as 1 min for *E. coli* O157:H7 and *L. monocytogenes*. *Salmonella* Typhimurium was better able to attach and grow on plums than was *E. coli* O157:H7, a finding in agreement with that of Barak et al. *(4)*. However, the same trend was not seen with peaches, on which *E. coli* O157:H7 was better able to attach and grow than was *Salmonella* Typhimurium. Barak et al. *(3)* and Jeter and Matthysee *(29)* found that *E. coli* O157:H7 and *Salmonella* produced fibrils and aggregative polymers for attachment. Plant pathogens produce similar fibrils to attach to plant hosts. Latham et al. *(33)* found that *Ruminococcus flavefaciens* attached to ryegrass after 30 min of exposure, *Pseudomonas lachrymans* attached to young cucumber leaves after 10 min *(34)*, and *Bacillus subtilis* attached to the surface of avocado leaves within 2 h *(18)*. Bacteria in the present study attached to one another, forming typical microcolonies. Barak et al. *(4)* also found that *S. enterica* used colonization niches on sprout surfaces, and cells attached to one another, therefore increasing the possible attachment surface area.

Foodborne pathogens used in this study colonized peach surfaces more effectively than plum surfaces, and this difference was attributed to the increased surface area of the peach due to the presence of trichomes. The trichomes on peaches provide a niche for bacteria, making detachment more difficult during washing. Trichomes also serve as additional colonization sites for microorganisms, thereby increasing the surface area that can be used for adherence, attachment, and eventual colonization. The colonization studies using the four selected foodborne pathogens indicated preferential attachment sites on the peach trichomes and nearby lenticels. Seo and Frank *(45)* found that *E. coli* O157:H7 and epiphytes that attached to the intact surface of lettuce leaves attached to areas located near stomata, on trichomes, and on veins. Takeuchi and Frank *(50)* found that plant pathogens may be better adapted than human foodborne bacterial pathogens to the phyllosphere. In the present study, *Salmonella* Typhimurium and *E. coli* O157:H7 were the least effective colonizers of stone fruit surfaces. *Salmonella* Typhimurium was able to produce microcolonies, but survival was poor. On cilantro leaves, *S. enterica* formed colonies 2 days postinoculation, and larger colonies were observed at 9 days postinoculation *(7)*. In the present study, *Salmonella* Typhimurium produced fewer microcolonies than did *S. aureus* and *L. monocytogenes* on the sections examined. Microcolony formation is one of the survival strategies used by bacteria cells to provide protection through the production of exopolysaccharides *(36)*.

Another important requirement for microbial colonization is successful reproduction. In this study, multiplication of all four foodborne bacterial pathogens was observed on the stone fruit surface areas, indicating that the bacteria were able to utilize nutrients available on the surface of the fruit. The increase in number of *S. aureus* cells also demonstrated this organism's ability to reproduce on peach surfaces. Colonization and survival of enteric bacteria has been noted on plants by various authors *(7, 25–27, 42, 48)*, mainly on leaves and roots.

After storage at the initial export temperature of 0.5°C, titers of all pathogens (except *S. aureus*) on peaches and plums decreased over time due to the inhospitable environmental conditions. Survival of foodborne pathogens can therefore be reduced by careful management and by maintaining export temperatures for stone fruit at 0.5°C. However, pathogen titers increased again once the fruit was removed from cold storage, simulating the export chain. Similarly, Francis and O'Beirne *(21)* found a decrease in titers of *E. coli* O157:H7 and *L. monocytogenes* when temperatures changed from 8 to 4°C. Most often, colonization is more successful at higher temperatures. Temperature and water activity play important roles in survival of bacteria on a plant surface. At high conducive temperatures (optimum temperatures) and high relative humidity, *S. enterica* was able to multiply rapidly in the phyllosphere *(7)*. The results of the present study indicate that *E. coli* O157:H7 inoculated onto stone fruit at realistic contamination loads will not survive the entire export chain when contaminated at the point of harvest when the correct cold chain regimens are followed. Survival of *E. coli* O157:H7 in this study was poor, even though the organism was able to adhere, attach, and colonize. Mitra et al. *(39)* found that *E. coli* spot inoculated onto spinach leaves followed the same trend, but Solomon et al. *(48)* found that the *E. coli* O157:H7 population declined but survived on lettuce seedlings for up to 30 days postinoculation. Temperature also influenced *E. coli* O157:H7 survival in the present study, with titers decreasing at ultralow temperatures (0°C) and slight recovery at higher (refrigeration) temperatures (for the high-level inoculation group).

L. monocytogenes and *S. aureus* survived on the stone fruit surfaces more effectively than did the other two pathogens studied. *L. monocytogenes* is able to survive freezing temperatures, and *S. aureus* can withstand a number of environmental stresses in its natural habitat (human skin). A fluctuation in bacterial numbers was observed from immediately after inoculation at 21°C, with a decrease in titer during storage at 12°C (peaches) or 7.5°C (plums). *L. monocytogenes* down-regulated attachment at 37°C; optimal colonization and survival occurred at 20°C, followed by 30°C and then 10°C *(23)*.

In the present study, *S. aureus* was unable to survive in high enough numbers to produce toxins when inoculated at a realistic level. However, when inoculated at an unnaturally high level the pathogen was able to survive and grow to populations large enough to potentially produce toxins. The likelihood of this scenario happening under natural circumstances is low, but these results shows the potential for the organism to maintain initial high titers. At the end of the simulated export chain, *E. coli* O157:H7 survived at titers that theoretically could lead to illness. This pathogen can cause disease at a level of 10^1 cells. *L. monocytogenes* survived at 10^3 cells, which may be high enough to cause illnesses *(52)*. In contrast, *S. aureus* populations must be at least 10^5 cells to produce toxins *(52)*. Future research should focus on the likelihood that contaminated fruit could cause consumer illnesses at the end of the supply chain.

In conclusion, for illness to result from the consumption of contaminated fresh produce, foodborne pathogens must adhere, attach, colonize, and proliferate to a level above the minimum infectious dose. When fresh produce is contaminated preharvest, the organism must survive through postharvest treatments, including export cold chain storage conditions. If the organism is able to survive on the fruit surface under export environmental conditions and then proliferate before consumption, the level of risk increases. *Salmonella* Typhimurium and *S. aureus* in pure culture inoculated onto fruit under laboratory conditions are low-risk foodborne pathogens on stone fruit. The results of this study indicate that *E. coli* O157:H7 and *L. monocytogenes* can survive at levels high enough above the minimum infectious dose on stone fruit surfaces under controlled conditions to be a food safety concern. However, these high levels were attained only when the fruits were artificially contaminated with an unnatural high inoculum load and not with a lower load. The time-temperature regimen therefore cannot suppress *E. coli* O157:H7 and *L. monocytogenes* at high levels on stone fruit, and preventative intervention strategies would therefore be required. However, it is unrealistic to assume that such artificially high levels of contamination would occur when basic good agricultural practices are followed.

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