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Food safety risks associated with the use of contaminated agricultural water in the production of table grapes

By

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Declaration

I declare that the dissertation hereby submitted to the University of Pretoria for the degree of Magister Institutions Agrariae (Plant Protection), has not been previously submitted by me for a degree at any other University.

Signature_____Date:_____

Musiiwa Morris Mahafha

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ABSTRACT

Food safety risks associated with the use of contaminated agricultural water in the production of table grapes

Supervisor: Prof. L. Korsten

Most agricultural water sources are often considered contaminated, due to poorly maintained sanitation systems, polluted river streams and other water catchment areas. Agricultural water used for irrigation and reconstitution of pesticides is suspected to play a direct or indirect role in the transmission of human pathogens to fresh produce. The contamination of fresh table grapes during pesticide spraying can therefore be seen as a potential risk factor. This study focuses on identifying possible sources and levels of bacterial contamination in a river, holding dam and tank in table grape production areas. In addition, the ability of selected pathogens to attach and survive on table grape surfaces was studied using transmission electron microscopy. Water sources sampled in this study were found to be microbiologically contaminated. Microbial populations varied with season, sampling period within a season and water treatment conditions. No human pathogens were detected under natural field conditions on crops irrigated with contaminated water used for reconstituting agricultural pesticides. This study further showed a wide range of pesticide products that permitted survival and multiplication of most of the tested foodborne pathogens i.e. *Escherichia coli* 157:H7, *Listeria monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus*. This information provides insight into the potential risk that may be associated with table grapes due to the use of contaminated water. These findings highlight the importance of considering pesticides used, water quality and spray schedules prior to application. Detailed risk assessment studies on the potential of contaminated irrigation water and the actual link with foodborne disease outbreaks have not been investigated and should in future be determined as well as intervention strategies.

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CHAPTER 1: GENERAL INTRODUCTION

Fresh water is a fundamental resource, integral to all ecological and societal activities including food and energy production, transportation, waste disposal, industrial development and human health (Gleick, 1993). Large amounts of water of varying quality are used in food production and processing and demand is likely to increase with time (Kirby *at al*., 2003). Globally, water continues to be a major sources of human diseases, -injuries and -death as many water resources remain unsafe (Sobsey, 2006; WHO, 2003). In South Africa, for example, fresh water is decreasing in quality because of municipal and industrial waste, deforestation and destruction of water catchment areas, urbanisation, human population growth and climatic change (Rand water, 2004).

Agricultural water is also highly contaminated with chemicals, textile substances or waste polluted with many pathogenic microorganisms (Cruan *et al*., 2006; Hamilton et al., 2006). Sources of agricultural water include rivers, dams, lakes and groundwater. Protecting these resources for agricultural use is however difficult (Koutsotoli *et al*., 2005; Izumi, 2008). In particular, agricultural water sources can harbour human pathogenic bacteria of public health concern such as *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., Vibrio cholerae and *Staphylococcus aureus* (Kistemann *et al*., 2002; Okafo, 2003; FBR, 2008). The main sources of agricultural water contamination may include faeces, sewage sludge, manure and industrial effluent (Beuchat, 2002; Johannessen *et al*., 2002; Canadian Horticultural Council. 2006). The quality of agricultural water is therefore of critical concern as it may spread and/or transfer foodborne pathogens to fresh produce (De Roever, 1998).

The World Health Organisation (WHO) reports over a thousand foodborne disease outbreaks annually. It is believed that millions of people suffer from diseases caused by contaminated food (WHO, 2008). Water like any food is a vehicle for the transmission of disease causing organisms and continues to

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cause significant disease outbreaks. It is estimated that the reported incidence of waterborne diseases represent less than 10 % of the real incidence. In developing countries, the WHO survey indicated that waterborne diseases may be 300 to 350 times more frequent than the reported cases (Department of Health, 2007; Department of Health, 2008). These diseases include bloody diarrhea, mild to severe intestinal discomfort, and severe dehydration to neurological symptoms (Scharff *et al*. 2009).

Fresh produce has been associated with the transmission of foodborne illness. Previous studies indicated the ability of foodborne pathogens to attach, survive and grow on or in raw produce increasing the potential health risk to consumers (Burnett & Beuchat, 2001; Beuchat, 2002). Foodborne pathogens may contaminate fresh produce at any stage through out the production cycle (De Roever, 1998). The possible sources of microbial contamination may include both pre- and post-harvest practices such as using infected soil, feces, inadequate composted manure, contaminated harvesting equipment, transport containers and processing equipment or product exposure to infected dust and insects (Beuchat, 2002; Johannessen *et al*., 2002; Canadian Horticultural Council. 2006). Furthermore, common agricultural practices such as using agricultural water (irrigation and water used in pesticides application) could possible introduce waterborne pathogens to fresh produce (fruit and vegetable) (Beuchat 2002; Guan *et al*., 2005; Ng 2005).

A number of waterborne disease outbreaks were associated with the consumption of fresh produce which are commonly consumed unpealed (tomatoes, apples) or uncooked (mangoes and oranges) (Buck *et al*., 2003; Izumi *et al*., 2008). Recently the European Union (EU) suffered from *E. coli* O14:H4 disease outbreak spread by contaminated cucumber and tomatoes produced from Germany (http://www.bbc.co.uk/news/world/europe-13683270). In South Africa, reporting of foodborne disease outbreaks is rare and information on the involvement of contaminated produce in illness is not

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available. This is probably due to the absence of food safety disease surveillance systems to detect and record such infections.

Fresh produce contaminated with waterborne pathogens represent a serious and costly health hazards in terms of sustainable food production and security (Blumenthal *et al*., 2000; Kirby *et al*., 2003). Table grapes like any other fresh produce rarely undergo minimal processing. To assure safety of table grapes in the supply chain, it is important to prevent microbial contamination during production and distribution. It is therefore, essential to study potential risks associated with current production practices to ansure that industry and the consumer are protected against adverse risks. The main objectives of this study are:

- To assess the microbial quality of water sources utilised by commercial table grape farmers;
- To determine the possible presence of waterborne pathogens on table grape surfaces if irrigated with contaminated water;
- To evaluate the *in vitro* impact of pesticide solutions on waterborne pathogens;
- To evaluate quality and safety of table grapes, thereby identifying food safety risk associated with the use of contaminated water to dilute pesticides;
- To determine the ability of waterborne pathogens to attach, survive, colonise and replicate on the surfaces of table grapes.

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1.2. REFERENCES

BBC News, 2011. E. coli outbreak: EU proposes 150m euros to aid farmers. (http://www.bbc.co.uk/news/world/europe-13683270). Accessed: 26 September 2011.

Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Journal of Microbes and Infection* 4: 413-423.

Blumenthal, U.J., Mara, D. D., Peasey, A., Ruiz-Palacios, G. & Stott, R., 2000. Guidelines for the microbiological quality of treated wastewater used in agriculture:Recommendations for revising WHO guidelines. *Bulletin of the World Health Organization* 78: 1104-1116.

Buck, J.W., Walcott, R.R. & Beuchat, L.R., 2003. Recent Trends in Microbiological Safety of Fruits and Vegetables. Plant Health Progress. www.cspinet.org/foodsafety/outbreak-alert.pdf. Accessed: 3 March 2008.

Burnett, S.L. & Beuchat, L.R., 2001. Foodborne pathogens: Human pathogens associated with raw produce and unpasteurised juices, and difficulties in contamination. *Journal of Industrial Microbiology and Biotechnology* 27: 104-110.

Canadian Horticultural Council, 2006. Leafy vegetables and cruciferae. On-farm food safety manual. Version 1 draft. Canadian Horticultural Council, Ottawa, Ontario, Canada. Accessed: 3 March 2009.

Craun, G.F., Calderon, R.L. & Wade, T.J., 2006. Assessing waterborne risks: an introduction. *Journal of Water and Health* 4: 1-16.

Department of Health., 2007. Draft: Clinical Guidelines on Management and Control of Infectious Foodborne Diseases in South Africa.

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www.doh.gov.za/docs/factsheets/guidelines/foodborne/part1.pdf. Accessed: 6 April 2010.

Department of Health. 2008. Guideline for environmental health officers on the interpretation of microbiological analysis data of food. www.doh.gov.za/docs/factsheets/microguide.pdf. Accessed: 6 April 2010.

De Roever, C., 1998. Microbiological safety evaluations and recommendations on fresh produce. *Food Control* 9: 321-347.

Gleick, P.H., 1993. Water and conflict fresh water resources and international security. *International Security* 18: 79-112.

Food and beverage report, 2008. Health, nutrition, hygiene. Curbing contamination.

Grabow, W., 1996. Water diseases: update on water quality assessment and control. *Water South Africa* 22: 193-203.

Guan, T.Y., Blank, G. & Holley R.A., 2005. Survival of pathogenic bacteria in pesticides solutions and on treated tomato plants. *Journal of Food Protection* 68: 296-304.

Hamilton, P.D., Gale, P. & Pollard, S.J.T., 2006. A commentary on recent water safety initiatives in the context of water utility risk management. *Environment International* 32: 958-966.

Izumi, H., Tsukada, Y., Poubol, J. & Hisa, K., 2008. On-Farm sources of Microbial Contamination of Persimmon Fruit in Japan. *Journal of Food Protection* 71: 52-59.

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Johannessen, G.S., Locarevic, S. & Kruse, H., 2002. Bacteriological analysis of fresh produce in Norway. *International Journal of Food Microbiology* 77: 199-204.

Kirby, R.M., Bartram, J. & Carr, R., 2003. Water in food production and processing: quantity and quality concerns. *Food Control* 14: 283-299.

Kistemann, T., Claben, T., Koch, C., Dangendorf, F., Fischeder, R., Gebel, J., Vacata, V. & Exner, M., 2002. Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. *Applied and Environmental Microbiology* 68: 2188-2197.

Koutsotoli, A.D. & Dimou, D.S., 2005. Long term and short term inducible effects of trifluralin on *Escherichia coli* and *Enterococcus faecalis. Microbial Ecology in Health and Disease* 17: 121-127.

Ng, J.P., Graham, H.F. & Gillian, M.H., 2005. Pesticides as a source of microbial contamination of salad vegetables. *International Journal of Food Microbiology* 101: 237-250.

Okafo, C.N., Umoh, V.J. & Galadima, M., 2003. Occurrence of pathogens on vegetables harvested from soils irrigated with contaminated streams. *The Science of the Total Environment* 311: 49-56.

Scharff, R.L., McDowell, J. & Medeiros, L., 2009. Economic Cost of Foodborne Illness in Ohio. *Journal of Food Protection* 72:128-136.

Sobsey, M.D., 2006. Drinking water and health research: a look to the future in the United States and globally. *Journal of Water and Health* 4: 17-21.

World Health Organisation, 2003. Eighth report of WHO surveillance program for control of foodborne infections and intoxications in Europe. http://www.who.int/foodsafety/publications/foodborne_disease/outbreaks. Accessed: 9 April 2010.

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World Health Organisation, 2008. Food disease outbreaks: Guidelines for investigation and Control. http://www.who.int/foodsafety/publications/foodborne_disease/outbreak_guidelines.pdf. Accessed: 9 April 2010.

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CHAPTER 2: LITERATURE REVIEW

Table grapes are important global traded fresh produce product. Food safety of fresh table grapes is of utmost importance in global trade to ensure compliance with voluntary and compulsory standards. Recent food safety case such as the *Escherichia coli* outbreak in the European Union (EU) has highlighted the importance of effective regulation, due diligence and sound scientific base for adequate risk assessment studies. This literature review provides an overview of the existing literature covering table grapes, food safety, waterborne pathogens and possible food safety risks to the industry and the consumer.

2.1. Historic perspective of table grapes

Grapes (*Vitis vinifera* L.) belong to the *Vitaceae* family and include table, wine and raisin grapes (Jackson, 1986b; Weaver, 1976). *Vitis vinifera* originated in the Caucasus Mountains between the Caspian and Black seas in central Asia Minor (Weaver, 1976; Jackson, 1986a). From Asia, grapes were introduced into other countries by tourists (Weaver, 1976). Cuttings of grape vines arrived in South Africa (SA) in 1652, in the colony of the then Cape of Good Hope (Jackson & Schuster, 1997).

2.1.1 Global table grape production

Grapes grow well throughout the year in most parts of the world where the mean temperature is between 10 and 20 ℃ (Jackson & Schuster, 1997). Italy is the largest producer of grapes followed by China, the United States of America (USA), Spain, and France, with SA at the tenth position (Appendix 1:Table 2.1). During the 2008/2009 growing season, world production of grapes was 67 million tones (m.t) when compared to other fresh fruit, and was in second position after apples at 68 m. t, followed by pears (21 m. t) and citrus (8 m. t) (Food and Agricultural Organisation, 2009). Chile, is the largest exporter of table grapes, followed by Italy, then the USA and, SA in the fourth

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place. China only exports small volumes of table grapes (Appendix 1: Table 2.1). Countries like China, USA, France and Germany form part of the top 15 importers of table grapes produce in the world (Appendix 1: Table 2.2 -) (Food and Agricultural Organisation, 2008).

2.1.2 South African table grape production

South Africa consists of many table grape production regions (14 010 ha) delivering a total production of 258 900 tones during the 2007/2008 season. The most important regions are Hex River Valley (33 %), Lower Orange River (29 %), Berg River (26 %), Limpopo Province (9 %) and Namaqualand (3 %). These production regions fall within climatic conditions which allow South African producers to produce grapes from November to May (South African Table Grapes Industry, 2008). South Africa is the leading country in the African continent in terms of grape production. During the 2007/2008 growing season, SA produced approximately 1.8 m. t of grapes obtained from 130 000 hectare of land with a yield of 137 818 kg/ha (FAO, 2008). South Africa exports approximately 84 % of table grapes to Europe and the United Kingdom (UK); and the remainder is destined for the Far East and Asia (9 %), Middle East and Mediterranean (5 %), America (1 %) and other parts of Africa (1 %) (Perishable Products Export Control Board, 2010).

2.2 Food safety

Food safety is a complex term, which means "*an assurance that food will not cause harm chemically, biologically or physically to the consumers when prepared, used or eaten according to its intended use*" (Foodstuffs, Cosmetics and Disinfectants Act 54, 1972). The recent global food scarcity resulted in an increased focus on food safety (Scholliers, 2008). A growing awareness amongst consumers and food industries emerged as the need for safe nutritious food increased (Raspor, 2007; Jevsnik *et al.*, 2008).

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Disease outbreaks linked to the consumption of contaminated food be it by physical, biological or chemical substances, creates major social and economic losses in communities and in national health systems (Anklam & Battaglia, 2001). Food contamination by infectious or toxic microorganisms can cause severe illness or even death if the food is improperly prepared or handled (Hugas *et al.*, 2007). Residues of agricultural chemicals in food and prolonged dietary exposure to such chemicals may pose long term adverse health effects on humans (Crutchfield, 1995). Foreign matters such as stones, metals, poisonous micronutrients, insects and other physical objects which do not form part of the food product can also pose a health risk if consumed (McLaughlin, 1999).

Agricultural products sold to the general public or traded internationally need to comply with the prescribed quality and food safety requirements and standards. If certain harmful microorganisms are detected on both fresh and processed agricultural produce, it can be rejected from both local and international markets. Resulting in the loss of income, and the concerned producer may face legal action (Agricultural Product Standards Act 119, 1990; Anklam & Battaglia, 2001). The recent outbreak of foodborne diseases in the EU highlights the importance of effective regulation, product recall, traceability and testing methods (http://www.bbc.co.uk/news/world/europe-13683270).

2.2.1 Quality and safety standards

Food safety concerns led to changes in the process of production, trade and distribution of food products. In response to these developments, both national and international governments adopted new regulations and enforce legislation to ensure continuous production of safe and quality food for all (Trienekens & Zuurbier, 2008). The World Trade Organisation- (WTO) with its government members apply sanitary and phytosanitay (SPS) measures and negotiate internationally to prevent technical barriers to trade and to enhance the implementation of food safety and bio-security measures. Private entities such as businesses and retailers require compliance with voluntary standards

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and certification systems based on Hazard Analysis Critical Control Points (HACCP) and International Standard Organization (ISO) systems from producers prior to going into conracted supplier agreements. Examples of some of these certification systems in the food safety area that are applicable in South Africa, include the British Retail Consortium (BRC), Globalgap, Tesco's Natures Choice, SANS 10330 and SANS 22000 standards. Some producers report compliance with standards being higher than those set by government (Holleran *et al.*, 1999; Fulponi, 2006).

Safety and quality control systems, standards and certification programmes are implemented to meet consumer expectations and assure protection of food industries (Holleran *et al.*, 1999). According to the World Health Organisation (WHO) these control systems and programmes achieve their objectives through protecting human, animal and plant health or life from disease causing organisms (DAFF, 2010).

2.3 Quality and safety of agricultural water

Fresh water resources are becoming increasingly scarce, driven by factors such as population growth, urbanisation, and global climate change (Kirby *et al.*, 2003). Contaminated water and food are major causes of mortality and malnutrition within human populations in many developing countries. Currently, food and water industries have a common objective of supplying quality and safe food to consumers (Marino, 2007). Sources and factors which contribute to the contamination of water include agricultural chemical, municipal and industrial wastewater discharge, mining activities and over loaded poorly maintained or leaking sanitation systems (WHO, 2000).

Besides chemical contaminats, agricultural water naturally contains many microorganisms (Hamilton *et al.*, 2006). Agricultural water can also be contaminated with a high concetration of pathogenic microorganisms which can cause diarrhoea and other serious infectious diseases (De Roever, 1998; Marino, 2007). A number of foodborne pathogens have been described as

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contaminants in agricultural water systems in reservoirs, canals, dams, furrows and rivers (Kirby *et al*., 2003).

Contaminated manure, dust or soil represents a risk and may spread pathogens in agricultural water that could end up in the food chain (Johannessen, 2002). In Canada, water was identified as a source of the worst outbreak of pathogenic bacteria i.e. *E. coli* O157:H7 (Kondro, 2000). In 2006, the USA reported 1 270 foodborne related disease outbreaks, 27 634 disease cases, 11 deaths and only 624 outbreaks with confirmed etiologies. *Salmonella* spp., *Listeria monocytogenes, Clostridium botulinum* and *E. coli* O157:H7 were responsible for 10 deaths and were also the most common attributed disease causal microorganisms (CDC, 2009). The general food commodities related to disease outbreaks were contaminated water, leafy and root vegetables, fruit and other ready to eat fresh produce. For example, in this instance vegetables resulted in 14 395 cases, fruit in 841 and other processed produce in 759 disease outbreaks (CDC, 2010). Foods may become sources of infection due to direct exposure to contaminated water and unclean environments (Marino, 2007).

2.3.1 Waterborne pathogens

Waterborne pathogens pose a major concern both to public health and the food industry, as food contamination by any human pathogen can lead to foodborne disease and rejection of products in the export market (Reilly & Kaferstein, 1997). Currently, there are more than 70 different foodborne pathogens that are believed to cause human illness (CDC, 2010). Foodborne- and waterborne- pathogens are microorganisms or agent (bacteria, viruses, protozoa and algae) that cause illness either through infections or production of toxic, and the illness occurs after the ingestion of contaminated food or water (Table 2.3 - Appendix) (National Research Council, 1996; Hamilton *et al*., 2006). According to Scharff *et al*. (2009), foodborne pathogens also impact on social issues such as pain, suffering and mortality.

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2.3.2. Waterborne pathogenic bacteria

Bacterial pathogens associated with water that cause most frequent disease outbreaks on fresh produce are *Salmonella enterica*, pathogenic *E. coli*, *Shigella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia* spp. and *Bacillus cereus* (Table 2.3) (Buzby *et al*., 1996; Brandl, 2006; Hamilton *et al*., 2006). Among the greatest concerns with human bacterial pathogens on fresh produce are enteric pathogens (*E. coli* 0157:H7 and *Salmonella* spp.) that have potential for growth prior to consumption or have a low infectious dose (Buck *et al*., 2003).

2.3.2.1. *Escherichia coli*

Escherichia coli is a gram negative and an important pathogen due to the gastroenteritis symptoms induced by the production of toxins while, the foodborne pathogens multiply and colonise the human gastrointestinal tract. The infectious dose of *E. coli* O157:H7 is as low as $10^1 – 10^2$ cells. However, even lower doses can cause illness in young children, the elderly and immune-compromised persons (Buzby *et al*., 1996; Cody *et al*., 1999).

Most of the reported outbreaks of *E. coli* O157:H7 illnesses have been associated with contaminated cattle products, such as undercooked beef and raw milk (Francis *et al*., 1999); and the most recent *E. coli* O104:H4 outbreak was associated vegetables (cucumber and tomatoes). Animal faeces are regarded to be the main source of *E. coli* contamination, but the pathogens are known to survive in soil, water (Izumi *et al*., 2008; Raspor, 2007), and in manure for more than 21 months (Jahannessesn *et al*., 2002). Low pH and high temperatures have been presumed to support the survival and growth of *E. coli* in most food products (De Roever, 1998). *Escherichia coli* can survive on the surface and inside fresh produce products such as strawberries, oranges, water melon, broccoli, cucumber and green peppers (Yu *et al*., 2001).

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2.3.2.2 *Salmonella* spp.

Salmonella spp. are Gram negative and the leading cause of foodborne outbreaks (Swamy *et al.,* 1996). Pathogenic *Salmonella* include Salmonella Typhimurium and Salmonella non-Typhimurium spp. that cause acute gastrointestinal illness such as gastroenteritis, organ focal infection, and systemic febrile infection. Normal levels necessary to cause salmonellosis range from 10^7 – 10^9 cells, but even a dose level of 10^5 cells may also cause food poisoning (Francis *et al*., 1999; Institute of Food Technologists, 2004).

Poultry and other products (eggs and dairy products) are the most commonly implicated sources in salmonellosis. However, soil and fresh produce can also become contaminated when coming into contact with faecal material, sewage and sewage polluted water which act as asource of *Salmonella* spp. (Brakett, 1999; Francis *et al*., 1999). *Salmonella* spp were isolated from leafy vegetables, beansprouts and salads [Center for Science in the Public interest (CSPI), 2004]. *Salmonella* spp. can survive and multiply at a low pH of 5 and temperatures of 22-24 ℃. Therefore, once produce has become contaminated, microorganisms can easily proliferate (WHO, 2001; Harris *et al*., 2003).

2.3.2.3 *Listeria monocytogenes*

Listeria monocytogenes is a Gram positive, rod shaped, facultative anaerobic and infectious bacterium (Rasmussen *et al*., 1991). The pathogens usually multiply inside human intestinal tracts, irritate the lining of the intestines, and cause human illnesses (Anonymous, 2000). Listeriosis is more severe in pregnant woman, newborn and adults with weakened immune systems (Buzby *et al*., 1996). Listeriosis causes symptoms such as nausea, vomiting, headache, fever, and severe infections like septicemia and meningitis In humans, ingestion of as few as 10^3 cells of the Listeria is high enough to cause illness and the symptoms may appear after 12 hours (Institute of Food Technologists, 2004; European Commision, 2002; Brandl, 2006).

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Most of the reported listeriosis outbreaks were associated with contaminated commercial processed food products such as vegetables (broccoli, carrots, cucumber, tomatoes and cabbage), milk, soft cheese and meat on which *Listeria monocytogenes* can survive due to its ability to proliferate at low temperatures (- 4 ℃) (Watkins & Sleath, 1981; Heisick *et al*., 1989). *Listeria monocytogenes* is considered ubiquitous in the environment, with the main source being soil, faeces, sewage, animals and man (Beuchat, 2002).

2.3.2.4. *Staphylococcus aureus*

Staphylococcus aureus is a Gram positive foodborne pathogen and it produces toxic substances that cause osteomylitis illness in human starting about 2-8 hours after the contaminated food is eaten. Other symptoms may include abdominal cramps, diarrhoea, vomiting, and sometimes headache and fever. Counts of 10^5 cells are high enough to cause food poisoning in humans (European Commision, 2002; CSPI, 2004).

Staphylococcal food poisoning occur more often in food which require hand preparation e.g. potato salad, cabbages, carrot, onions and lettuce ham salad and sandwich spreads (CDC, 2000; CSPI, 2004). Infected wounds, lesions, and boils of food handlers may also be sources of contamination, as well as coughing and sneezing by individuals with respiratory infections. A temperature of 20-22 ℃ to allow the growth of *S. aureus* and increases production of toxin, but the viability of these cells decreases markedly when frozen (Spasford *et al*., 2004).

2.4. Waterborne pathogens and fresh produce

Fresh produce has been linked with the transmission of several foodborne pathogens. Contamination of fresh produce by waterborne pathogens occurs during production, harvesting and processing (De Roever, 1998). Due to changes in dietary habits there has been an increase in consumption of fresh,

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minimally processed fruit and vegetables (Beuchat, 2002; Buck *et al*., 2003). Produce related illnesses has also been enhanced by changes in production, processing methods, sources of fresh produce and emergence of new foodborne pathogens not previously important in raw produce (Ng *et al*., 2005; Izumi *et al*., 2008). Furthermore, the increase in international travel and trade modification and adaptation of foodborne pathogens in the food production system, as well as human demographics and behaviour have also contributed to the increase in foodborne disease threats (WHO, 2002).

Outbreaks of waterborne illness caused by ingestion of contaminated fruit and vegetables have been documented for centuries (De Roever, 1998). Cases of *Escherichia coli* and *Salmonella* spp. were reported in association with the consumption of alfalfa, celery, lettuce, tomatoes, radish, apples, mangoes and oranges (Buck *et al*., 2003; Izumi *et al*. 2008). In countries like England and Wales, fresh produce caused 6.4% to 10.1% of all outbreaks in the period between 1993 and 2000 (WHO, 2000; WHO, 2003). In 1996, *E. coli* O157:H7 alone caused the largest bacterial enteric disease outbreak in Japan with more than 6 000 cases that were linked to the consumption of contaminated radish sprouts (Yu *et al*., 2001).

Escherichia coli were also reported to be responsible for large outbreaks of bloody diarrhea in Southern Africa (Swaziland) after exposure to contaminated water and consumption of fresh produce (Scheutz & Samuelsson, 2001). On the other hand, human listeriosis has been epidemiologically linked to the consumption of fresh cabbage and lettuce (Beuchat, 1995). Staphylococcal food poisoning outbreaks occurred more often in food which requires hand preparation e.g. potato salad, ham salad and sandwich spreads (CDC, 2000; CSPI, 2004).

2.5. The use of crop protection remedies on agricultural crops

Plants are directly or indirectly the main source of food in the world. There are 80 000 to 100 000 diseases caused by plant pathogens which affect growth,

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yield and quality (Agrios, 2005). Plants compete with 30 000 species of weeds of which 18 000 species cause serious economic losses. More than 30 000 species of nematodes attack plants and about 10 000 plant eating species of nematodes add to devastating losses of crops world wide (Ware, 1978; Agrios, 2005).

One-third of the world's food crops are destroyed by pests during growth, harvest and storage with losses being higher in most emerging countries (CODEX Alimentarius Commission, 2007). Crop protection remedies can protect crops and save farmers money and increase profit margins by preventing crop losses due to insects and other pests. Not using crop protection products can result in crop yields being reduced by 31 to 40% (Agrios, 2005).

In the last 100 years, control of plant diseases and other plant pests have depended increasingly on the extensive use of crop protection remedies (Agrios, 2005). Crop protection remedies have been developed to protect the crop during various stages of growth and after harvest and thereby improving quality and quantity of agricultural products worldwide (McEwen, 1997). It is expected that these products will continue to play a vital part in the safety and economic production of food in the foreseeable future (Guan *et al*., 2001). In most parts of the developed world, pesticides are used as an essential component in integrated pest management programmes (Wilson, 2003). In economic terms, for the $3 billion invested in remedy control, about $12 billion are returned in increased crop yield, which represent an excellent return on investment (Agrios, 2005).

2.5.1. Non-target effect of crop protection remedies on waterborne pathogens

Crop protection products raise a number of environmental concerns. Over 98% of sprayed insecticides and fungicides and 95% of herbicides reach a destination other than their target species. Destinations such as humans,

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beneficial plants and non target insect species, air, soil, water, bottom sediments, and food (Ware, 1978; Pimentel & Levitan, 1986). Human poisonings also represent a high price to pay for crop protection products used. An estimated 45 000 total human poisonings occur annually, including about 3 000 cases admitted to hospitals and 200 recorded deaths (Amoah *et al*., 2006).

In most countries, remedies for sale and in use must be approved by a governmental agency. Studies must be conducted to indicate whether the material is safe to use and effective against the intended pest (CODEX Alimentarius Commission, 2007). During the registration process, a label is created which contains directions for the proper use of the remedies. Based on acute toxicity, all registered remedies are assigned to a maximum residue limit, handling precautions and a toxicity class (Appendix 1: Table 2.5) (Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 36 0f 1947; Elson & Biehn, 1986; CODEX Alimentarius Commission, 2007).

2.5.2. Crop protection remedies and waterborne pathogenic bacteria

Except for the active ingredients, remedies consist of other inert products such as, surfactants, emulsifier, dispersants, solubilisers, antifoamers/defoamers, campatabilisers, solvents, carriers, wetting agents, and other preservatives. All these additives and adjuvants are added to increase the effectiveness and stability of chemical products (Guan *et al*., 2001; Guan *et al*., 2005; Ng *et al*., 2005).

A number of crop protection products have been shown to stimulate the growth and survival of some foodborne pathogenic bacteria (Coghlan, 2000; Guan *et al*., 2001; Ng *et al*., 2005). Guan *et al.* (2001), found that *E. coli* O15:H7, *Salmonella* spp., *Shigella* spp., and *Listeria* spp. survived in the following pesticide solutions: Alfolan, Bravo 500, Lorsban 4E and Ambush. The inert ingredients have been shown to deteriorate if the pesticides solution is stored for longer periods than the identified shelf life, which then allows for

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the multiplication of the pathogen. Therefore, it is advisable to adhere to the expiry date or apply products immediately after preparation. Inert additives allow pathogens to survive and proliferate because they provide nutrients for growth (Guan *et al*., 2005).

2.6 Conclusion

The recent increase in fresh produce production and consumption is accompanied by ever changing agricultural practices that could play a direct or indirect role in the transmission of foodborne pathogens from the gate to the table. Contamination of produce can occur in the orchard; during harvesting, postharvest handling, processing, transporting and marketing or at home. Contamination of table grapes and other fresh produce by waterborne pathogens may have negative effects on commercial viability and creditability for the producer and on the health of the consumer. Since most the pathogenic bacteria are waterborne and can survive well in water sediments, sources of agricultural water should be added to the risk factors of potential crop contamination.

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2.7 References

Agricultural Product Standards Act No. 119 of 1990. www.daff.agric.za/docs/plantquality/ foodsafety.html. Accessed: 23 April 2008.

Agrios, G.N., 2005. Introduction. In: Plant Pathology. 5th Edition. p. 4-7. Published by Elsevier Academic Press, New York, USA.

Amoah, P., Drechsel, P., Abaidoo, R.C. & Ntow, W.J., 2006. Pesticides and pathogen contamination of vegetables in Ghana's Urban Markets. *Archives of Environmental Contamination and Toxicology* 50: 1-6.

Anklam, E. & Battagilia, R., 2001. Food analysis and consumer protection. *Trends in Food Science and Technology* 12: 197-202.

Anonymous, 2000. Pathogens and produce: Foodborne putbreaks/incidents. european chilled food federation. Presented to the EC Scientific Committee for Food. www.blackwell-synergy.com/doi/full. Accessed: 3 March 2008.

BBC News, 2011. E. coli outbreak: EU proposes 150m euros to aid farmers. (http://www.bbc.co.uk/news/world/europe-13683270). Accessed: 26 September 2011.

Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Journal of Microbes and Infection* 4: 413-423.

Brackett, R.E., 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. *Post-harvest Biology and Technology* 15: 305-311.

Brandl, M.R., 2006. Fitness of human enteric pathogens on plants and implications for food safety. *The Annual Review of Phytopathology* 44: 367-392.

© University of Pretoria

Buck, J.W., Walcott, R.R. & Beuchat, L.R., 2003. Recent trends in microbiological safety of fruits and vegetables. Plant health progress. www.cspinet.org/foodsafety/outbreak-alert.pdf. Accessed: 3 March 2008.

Burnett, S.L. & Beuchat, L.R., 2001. Foodborne pathogens: Human pathogens associated with raw produce and unpasteurised juices, and difficulties in contamination. *Journal of Industrial Microbiology and Biotechnology* 27: 104-110.

Buzby, J.C., Roberts, T., Jordan Lin, C.T. & MacDonald, J.M., 1996. Bacterial foodborne disease: Medical dosts and productivity losses. Food and consumer economics division, economic research service, U.S. Department of Agriculture. Agricultural Economic Report No. 741. www.ers.usda.gov/Publications/AER741/. Accessed: 3 March 2008.

Centers for Disease Control and Prevention, 2000. Surveillance for foodborne-disease outbreaks United States, 1993–1997. *Morbidity and Mortality Weekly Report* 49: 1-51.

Centers for Disease Control and Prevention, 2006. Surveillance for foodborne disease outbreaks - United States, 1998 - 2002. *Morbidity and Mortality Weekly Report* 55: 1-34.

Centers for Disease Control and Prevention, 2009. Surveillance for foodborne disease outbreaks - United States, 2006. *Morbidity and Mortality Weekly Report* 58: 609-615.

Center for Science in the Public Interest, 2004. Outbreak alert! Closing the gaps in our federal food safety net. Washington, D.C. http://www.cspinet.org/Report. Accessed: 3 March 2008.

CODEX Alimentarius Commission, 2007. Proposed draft guidelines for the maximum residue limits for permissible chemicals used on Grapes.

© University of Pretoria

Washington, DC, CODEX Committee on Food Hygiene, CODEX Alimentarius Commission, Version 2. 1-5.

Cody, S.H., Glynn, K.M., Farrar, J.A., Cairns, K.L., Griffin, P.M., Kobayashi, J., Fyfe, M., Hoffman, R., King, A.S., Lewis, J.H., Swaminathan, B., Bryant, R.G. & Vugia, D.J., 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. *Annals of Internal Medicine* 130: 202-209.

Coghlan, A.C., 2000. Food poisoning bugs in crop spray. *New Scientist*: 20.

Crutchfield, S.R., 1995. Economic issues associated with food safety. United States Department of Agriculture Economic Research Service. 137-147.

De Roever, C., 1998. Microbiological safety evaluations and recommendations on fresh produce. *Food Control* 9: 321-347.

De Waal, C.S., Johnson, K. & Bhuiya, F., 2007. Outbreak alert! 2006. Center for science in the public interest. www.cspinet.org/foodsafety/outbreak-alert.pdf. Accessed: 3 March 2008.

Elson, J.E. & Biehn, W.L., 1986. Methods of evaluating pesticides for control of plant pathogens. Pages 1-2. In: Hickey. K.D (Eds). Fungicides and nematicides testing and pesticides registrations. Interregional research project No. 4, Cook Collage, Rutgers University, New Brunswick.

Environmental Protection Agency, 1999. Environmental regulations and technology. Control of pathogens and vector attraction in sewage sludge. U.S. Environmental Protection Agency. EPA/625? R-92-013.111. www.epa.gov/nrmrl/pec/bsguidance.html. Accessed: 23 April 2008.

European Commission, 2002. Risk profile on the microbiological contamination of fruits and vegetables eaten raw. Report of the scientific

© University of Pretoria

committee on food. www.europa.eu.int/comm/food/fs/sc/scf/index_en.html. Accessed: 4 March 2008.

Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act No. 36 of 1947. www.daff.agric.za. Accessed: 23 April 2008.

Food and Agricultural Organisation, Statistics Division, 2009. Major food and agricultural commodities and producers of deciduous fruits: faostat.fao.org/site/339/default.aspx. Accessed: 27 September 2011.

Food and Agricultural Organisation, Statistics Division, 2008. Export and Import of Grapes: faostat.fao.org/site/342/default.aspx. Accessed: 8 December 2010.

Foodstuffs, Cosmetics and Disinfectants Act No. 54 of 1972. www.nda.agric.za/docs/ plantquality/foodsafety.htm. Accessed: 23 April 2008.

Francis, G.A., Thomas, C. & O'Beirne, D., 1999. The microbiological safety of minimally processed vegetables. *International Journal of Food Science and Technology* 34: 1-22.

Fulponi, L., 2006. Private voluntary standards in the food system: The perspective of major food retailers in OECD countries. *Food policy* 31: 1–13

Guan, T.Y., Bank, G., Ismond, A. & Van Acker, R., 2001. Fate of foodborne bacterial pathogens in pesticide products. *Journal of the Science of Food and Agriculture* 81: 503-512.

Guan, T.Y., Blank, G. & Holley, R.A., 2005. Survival of pathogenic bacteria in pesticides solutions and on treated tomato plants. *Journal of Food Protection* 68: 296-304.

© University of Pretoria

Hamilton, P.D., Gale, P. & Pollard, S.J.T., 2006. A commentary on recent water safety initiatives in the context of water utility risk management. *Environment International* 32: 958-966.

Harris, L.J., Farber, N.J., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H. & Busta, F.F., 2003. Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and fresh cut produce. *Comprehensive Reviews in Food Science and Food safety* 2: 78-141.

Heisick, J.E. Wagner, M.L., Nierman, M.L. & Peeler, J.T., 1989. *Listeria* spp. found on fresh market produce. *Applied and Environmental Microbiology* 55: 1925-1957.

Hobbs, J.E., Fearne, A. & Spriggs, J., 2002. Incentives structures for food safety and quality assurance: an international comparison. *Food Control* 13: 77-81.

Holleran, E., Brendahl, M.E. & Zaibet, L., 1999. Private incentives for adopting food safety and quality assurance. *Food Policy* 24: 669-683.

Hoxie, N.J., Davis, J.P., Vergeront, J.M., Nashold, R.D. & Blair, K.A., 1997. Cryptosporidiosis-associated mortality following a massive waterborne outbreak in Milwaukee, Wisconsin. *American Journal of Public Health* 8: 2032-2035.

Hugas, M., Tsigarida, E., Robinson, T. & Calistri, P., 2007. Risk assessment of biological hazards in the European Union. *International Journal of Food Microbiology* 120: 131-135.

Institute of Food Technologists, 2004. Bacteria associated with foodborne diseases. http://members.ift.org/NR/rdonlyres/3DEA7A91-DF48-42CE-B195-06B01C14E273/0/bacteria. pdf. Accessed: 10 April 2010.

© University of Pretoria

Izumi, H., Tsukada, Y., Poubol, J. & Hisa, K., 2008. On-farm sources of microbial contamination of persimmon fruit in Japan. *Journal of Food Protection* 71: 52-59.

Jackson, D., 1986a. The distribution of fruits. In: Temperate and subtropical fruits production. Pages 1-7. Butterworths, Wellington, New Zealand (Ltd).

Jackson, D., 1986b. Grapes. In: Temperate and subtropical fruits production. Pages 197-203. Butterworths, Wellington, New Zealand (Ltd).

Jackson, D. & Schuster, D., 1997. The geographical distribution of the grapes. In:The production of grapes and wines in cool climates. Pages 3-48. Lincoln University Press, New Zealand (Ltd).

Jevsnik, M., Hlebec, V. & Raspor, P., 2008. Consumers' awareness of food safety from shopping to eating. *Food Control* 19: 737-745.

Johannessen, G.S., Locarevic, S. & Kruse, H., 2002. Bacteriological analysis of fresh produce in Norway. *International Journal of Food Microbiology* 77: 199-204.

Kirby, R.M., Bartram, J. & Carr, R., 2003. Water in food production and processing: quantity and quality concerns. *Food Control* 14: 283-299.

Kondro, W., 2000. *E. coli* outbreak deaths spark judicial inquiry in Canada. *The Lancet* 355: 2058.

Koutsotoli, A.D. & Dimou, D.S., 2005. Long term and short term inducible effects of trifluralin on *Escherichia coli* and *Enterococcus faecalis. Microbial Ecology in Health and Disease* 17: 121-127.

© University of Pretoria

Marino, D.D., 2007. Water and food safety in the developing world: Global implications for health and nutrition of infants and young children. *Journal of American Diet Association* 107:1930-1934.

McEwen, F.L., 1997. Pesticide management and insecticide resistance. In: Pesticides residue and agriculture workers – An overview. Page 37. In: D.L. Watson & A.W.A. Brown (Eds). Academic Press, New York, USA.

McLaughlin, M.J., Parker, D.R. & Clarke, J.M., 1999. Metals and micronutrients – Food Safety issues. *Field Crops Reaserch* 60: 143-163.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V., 1999. Food-related illness and death in the United States. *Emerging Infectious Diseases* 5: 607-625.

National Department of Agriculture, Fisheries and Forestry, 2008. List of registered pesticides and their maximum residue limits for grapes. www.nda.agric.za. Accessed 23 April 2008.

National Research Council, 1996. Public health concerns about infectious disease agents. In use of reclaimed water and sludge in food crop production, National Research Council, National Academic Press. www.nap.edu/books/0309054796/html/index.html. Accessed: 23 April 2008.

Ng, J.P., Graham, H.F. & Gillian, M.H., 2005. Pesticides as a source of microbial contamination of salad vegetables. *International Journal of Food Microbiology* 101: 237-250.

Nguz, K., 2007. Assessing food safety system in Sub-Saharan countries: An overview of key issues. *Food Control* 18: 131-134.

Nystrom, B., Bjornsater, B. & Blanck, H., 1999. Effects sulfonylurea herbicides on non-target aquatic microorganisms growth inhibition of micro algae and

© University of Pretoria

short term inhibition of adenine and thymidine incorporation in periphyton communities. *Aquatic Toxicology* 47: 9-22.

Perishable Products Export Control Board, 2009. Section 2, Perishable export products. In: PPECB Export Directory. Seventh Edition. Pages 2-25. Malachite Design & Publisher, Cape Town, South Africa.

Perishable Products Export Control Board, 2009. List of MRL's attachment extracted from EUREPGAP MRL Guideline. ww.ppecb.com/.../SurveyofexistingMRL.InformationandSystemsWebsiteVer.xls. Accessed 16 April 2010.

Pimentel, D. & Levitan, L., 1986. Pesticides: Amounts applied and amounts reaching pests. *Biological Science* 36: 86-91.

Rasmussen, O.F., Beck, T., Olsen, J.E., Dons, L. & Rossen, L., 1991. *Listeria monocytogenes* Isolates can be classified into two major types according to the sequence of the Listeriolysin gene. *Infection and Immunity* 59: 3945-3951.

Raspor, P., 2007. Total food chain safety: how good practices can contribute? *Trends in Food Science and Technology* 19: 405-412.

Reilly, A. & Kaferstein, F., 1997. Food safety hazards and the application of the hazard analysis and critical control point (HACCP) system for their control in aquaculture production. *Aquaculture Research* 28: 735-752.

Roberts, T. & Smallwood, D., 1991. Data needs to address economic issues in food safety. *American Journal of Agricultural Economics* 73: 933-942.

Sanders, W.E. Jr. & Sanders, C.C., 1997. Enterobacter spp. Pathogen poised to flourish at the turn of the century. *Clinical Microbiology Reviews* 10: 220-241.

© University of Pretoria

Scheutz, F. & Samuelsson, S., 2001. Verocytotoxin producing *E. coli* in Denmark 1997-2000. Epidemiology News No. 25. www.microbionet.com.au/vtecfullref3u.htm. Accessed: 25 April 2008.

Scharff, R.L., McDowell, J. & Medeiros, L., 2009. Economic cost of foodborne illness in Ohio. *Journal of Food Protection* 72: 128-136.

Scholliers, P., 2008. Defining food risks and food anxieties throughout history. *Research Report: Appetite* 51: 3-5.

South African Table Grapes Industry. Grape producer. www.sgati.co.za/site/producers. Accessed: 2 March 2008.

Spasford, K.E., Rasooly, A., Taitt, C.R. & Ligler, F.S., 2004. Detection of *Campylobacter* and *Shigella* species in food samples using an Array Biosensor. *Analytical Chemistry* 76: 433-440.

Swamy, S.C., Barnhart, H.M., Lee, M.D. & Dreesen, D.W., 1996. Virulence determinants *invA* and *spvC* in salmonellae isolated from poultry products, wastewater, and human sources. *Applied and Environmental Microbiology* 62: 3768-3771.

Thomson, K.S., Sanders, C.C. & Moland, E.S., 1999. Use of microdilution panels with and without β-Lactamase Inhibitors as a phenotypic test for β-Lactamase production among *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii*, and *Serratia marcescens. Antimicrobial Agents and Chemotherapy* 43: 1393-1400.

Trienekens, J. & Zuurbier, P., 2008. Quality and safety standards in the food industry, developments and challenges. *International Journal of Production Economics* 113: 107-122.

© University of Pretoria

United States Department of Agriculture-Foreign Agricultural Service (USDA-FAS) 2005. World table grapes and outlook, www.fas.usda.gov/table grapes. Accessed: 3 March 2008.

United States Department of Agriculture-Foreign Agricultural Service, 2008. World table grapes and outlook, www.fas.usda.gov/table grapes. Accessed: 3 March 2008.

Ware, G.W., 1978. Pesticides: Chemical tool. Pages 3-15. In: Pesticides theory and application. W.H. Freeman and Company. USA.

Watkins, J. & Sleath, K.P., 1981. Isolation and enumaration of *Listeria monocytogenes* from sewage, sewage sludge and river water. *Journal of Applied Bacteriology* 50: 1-9.

Weaver, J.W., 1976. History and classification of grapes. Pages 3-9. In: Grape growing. Edited by A Wiley Interscience Publication. New York: U S A.

Wilson, M.F., 2003. Introduction. Pages 1-6. In: Optimizing pesticide use. Edited by John Wiley and Son, Ltd. UK.

World Health Organisation, 2000. Seventh report of WHO surveillance program for control of foodborne infections and intoxications in Europe. FAO/WHO Collab. Cent. Res. Train. Food hygiene zoonoses. www.who.int/foodsafety/publications /foodborne_disease/en. Accessed: 23 April 2008.

World Health Organisation, 2001. Surveillance programme for control of foodborne infections and intoxifications in Europe. *News Letter* No. 70. www.who.int/foodsafety/publications/foodborne_disease/en. Accessed: 23 April 2008.

© University of Pretoria

World Health Organisation, 2002. Foodborne diseases, emerging. Fact sheet no. 124. http://www.who.int/mediacentre/factsheets/fs124/en/print.html. Accessed: 13 April 2010.

World Health Organisation, 2003. Eighth report of WHO surveillance program for control of foodborne infections and intoxications in Europe. Collab. Cent. Res. Train. Food Hygiene Zoonoses. www.who.int/foodsafety/publications/foodborne_disease/en. Accessed: 23 April 2008.

World market and trade, www.fas.usda.gov/table grapes. Accessed: 3 March 2008.

Yu, K., Newman, M.C., Archbold, D.D. & Hamilton, T.R., 2001. Survival of *Escherichia coli* 0157:H7 on strawberry fruit and reduction by chemical agents. *Journal of Food Protection* 64: 1334-1340.

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Chapter 3: Microbial quality of on farm water sources and fate of *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* in pesticides

3.1 Abstract

Agricultural water sources are known to be contaminated with various waterborne pathogens. The potential of these pathogens to be transferred to fresh produce during pesticide spraying can be regarded as a potential health risk to consumers. On farm water sources i.e. river, holding dam and pesticides spray mixes were analysed to quantify microbial load and detect presence of waterborne pathogens. Though water sources were considered contaminated, levels of presumptive pathogens detected were relatively low. The *in vitro* effect of 20 pesticides on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* survival, or growth was also evaluated. Sterile water was used to reconstitute pesticides at recommended dose before 10^4cfu/ml of pathogens was added. Following various time intervals of 0, 1, 4 and 8 hours of incubation at 20-22℃, *E. coli* O157:H7*, S. enterica* subsp. *enterica* Typhimurium*, L. monocytogenes* and *S. aureus* survived in Flint 50 WG, Strobin, Rovral aquaflow, Teldor 500 SC, Dipel DF, Azinophosmethyl, Cypermethrin and Thioflo. *Staphylococcus aureus* and *L. monocytogenes* died immediately in Sulphur and Kumules, while Prosper instantly inhibited the survival of *L. monocytogenes* and *S.* Typhimurium. Brilliant SL and Erador solutions generally permitted the increase of the test pathogens after 8 hours incubation. No viable count could be found with Copper hydroxide after 4 hours incubation. Dithane decreased *S. aureus,* while the other pathogens under study were inhibited when exposed for 4 and 8 hours. This study shows the potential of some pesticide used in the contribution of food safety risks. However, this highly depends on the contamination level of the sources of water.

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3.2 Introduction

Fresh produce has increasingly been linked with the transmission of foodborne pathogens resulting in increased disease outbreaks. The main source of contamination in these cases has been associated with poor pre-harvest practices such as using polluted irrigation water or contaminated manure (De Roever, 1998; Izumi *et al*., 2008). Contaminated fresh produce pose potential risks to the public health, as it may lead to various foodborne illnesses and disease outbreaks, and the entire food industry (Guan *et al*., 2001).

The Center for Disease Control and Prevention reported that, in the United States of America (USA) alone, 76 million foodborne infections are reported annually (Mead *et al*., 1999). Of the 41 % of the cases originate from fruit, vegetable or juice contaminated with pathogenic bacteria (Guan *et al*., 2001). Bacterial pathogens that are often associated with disease outbreaks from fresh produce include *Salmonella enterica*, pathogenic *Escherichia coli*, *Shigella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia* spp. and *Bacillus cereus* (Buzby *et al*., 1996; Brandl, 2006). The most important pathogens on fresh produce are enteric foodborne pathogens (*E. coli* O157:H7 and *Salmonella* spp.) due to their growth potential prior to consumption and their low known infectious dose (Buck *et al*., 2003). In 1996, the largest enteric disease outbreak of *E. coli* 0157:H7 linked to consumption of contaminated radish sprouts occurred in Japan and caused more than 6 000 cases of food poisoning (Yu *et al*., 2001). Countries like England (De Roever, 1998) and the USA (Zhao *et al*., 1993; De Waal *et al*., 2007) have previously reported on disease outbreaks caused by *E. coli* O157:H7, where infections were linked to the consumption of contaminated apple and apple products. In May 2011, the European Union experienced the worst foodborne disease outbreak caused by a virulent verocytotoxin producing *E. coli* O104: H4 isolated from cucumber and tomatoes from Germany. Due to some implication the outbreak was linked to produce from

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Spain and the Spanish vegetable producers lost €225 million per week (http://www.bbc.co.uk/news/world-europe-13683270).

The use of contaminated water has long been related to malnutrition, human illness, injuries and death (World Health Organisation, 2003). Contaminated water can directly cause disease outbreak, e.g. in Canada, water was identified as a source of the worst outbreak of pathogenic bacteria *E. coli* O157:H7 (Kondro, 2000). After irrigating tomato fields with contaminated water, a multistate disease outbreak associated with *Salmonella* infection in 1990, 1993 and 1999 occurred in the USA (Hedberg et al., 1999; Cummings et al., 2001).

Pesticides have contributed to the improved the quality and quantity of agricultural products; and will continue to play a vital part in the economic production of food in future (McEwen, 1997). Prior to pesticide application, agricultural water is used to mix the chemical to obtain the desired final concentration (Guan *et al*., 2001; Ng *et al*., 2005). The main sources of water used on farms are rivers, dams, lakes, boreholes, and streams (Nystrom, 1999; Koutsotoli *et al*., 2005). Faecal materials, soil and other inputs such as sewage overflow and introduced foodborne pathogenic bacteria in water sources can affect quality and safety of agricultural water (Kirby, 2003).

Microbial status of farm water sources used for preparation of plant protection remedies should be considered prior to application of pesticides (Ng *et al*., 2005). Studies by Guan *et al*. (2001) and Coghlan (2000), showed that dilution of various commercial plant protective products with contaminated water may present suitable conditions for the survival and growth of pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp. and *E. coli* O157:H7. Furthermore, other studies confirmed that pesticide solutions prepared using contaminated water could result in the spread of foodborne pathogens to the crops cultivated in the field, especially if the pesticide solutions support microbial growth (Guan *et al*., 2005). It was concluded that once prepared, delay in using pesticide spray mixes is critical

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as it could allow the number of bacterial cells already in the water to increase (Guan *et al*., 2001; Ng *et al*., 2005).

Therefore, agricultural water could be a source or critical factor in spreading and transferring foodborne pathogens to fresh produce. The effect of most registered pesticides used in the South African table grapes industry has not been determined for foodborne pathogenic bacteria. The aim of this study was therefore to evaluate microbiological load and to determine the presence of *Escherichia coli* O157:H7, *Salmonella enterica* subsp. *enterica* serotype Typhimurium, *Listeria monocytogenes* and *Staphylococcus aureus* in water sources used during the production processes. In the study, selected pesticides were evaluated to confirm the inhibition, survival or multiplication of *E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium and *S. aureus.*

3.3 Material and Methods

3.3.1 Sample collection

Water samples were collected from single farm in the Western Cape that is producing table grapes for the local and export markets. Agricultural water was sampled at the edge and upper layer of both holding dam and rivers that are used for irrigation and mixing of pesticides. The experiment was carried out over two seasons (2008/2009 season and 2009/20010 season). For each season, two different trials were conducted i.e. early- and mid season. From each sampling point, water was collected in sterile five litres and one-hundred millilitre plastic bottles. In both trials, sample of spray solution was also collected. Spray solution of the first trial consists of Dithane and Teldor 500 SC, while only Teldor 500 SC was used in the second trial. All samples were transported to the laboratory, at the University of Pretoria for analysis.

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3.3.2 Water analysis

From each sampling point, five replicates of one litres were analysed for the microbial viable count (bacterial, fungal and yeast). Each one litre of water was filtrated through cellulose nitrate filters of 0.45 µm pore size (Sartorius, Goettingen, Germany). Each filter paper was transferred to 9 ml of tryptone soy broth (TSB). After the mix, one millilitre of TSB solution was transferred to a 9 ml of Ringers solution to set up a dilution series. Solutions were then plated out in duplicate onto Standard 1 agar (STD 1) and Malt Extract Agar (MEA) and incubated for 7 days at 25 ℃. Colony count was done and data was recorded for statistical analysis.

The TSB solutions were incubated in a shaking incubator for 48 hours at 37 ℃ before DNA extraction was done following 1% Triton X-100 DNA extraction protocol. Low level of the isolated potential foodborne pathogens were identified and confirmed using a multiplex Polymerase Chain Reaction (PCR). A 1.5 µl sample of the supernatant was used as a template for each PCR. Each 25 µl PCR reaction mixture contained 0.3 µl if BioTaq polymerase (5U/ µl), 1.5 µl $MgCl_2$ (50 mM), 0.75 µl dNTPs (10 mM of each), 2.5 µl NH_4 reaction buffer (10x) (all from Bioline, Celtic Molecular Diagnostics, Cape Town, South Africa), 1.75 µl Bovine Serum Albumin acetylated (10 mg/ml) (Promega, Madison, United States of America), 1.25 µl Dimethyl Sulfoxide (Saarchem, Merck) and 0.3 µl of each primer (Whitehead Scientific, Cape Town). Primers used in this study are listed in table 3.1. For control purposes, a PCR reaction mixture containing sterile double distilled water and all other reagents except the DNA template was included. Thermocycling was performed using a Eppendorf Thermocycler (Merck, Johannesburg, South africa) and the PCR were as follows: 94 ℃ for 2 min, followed by 30 cycles of 94 ℃ for 30 s, 61 ℃ for 45 s, 72 ℃ for 1.5 min, with the final extension at 72 ℃ for 7 min. PCR products were visualised following gel electrophoresis on 1 % agarose gels (Wang and Slavik, 2005).

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Five replicates for each sampling point were analysed in a Colilert 18®-test to determine the presence/absence of total coliforms/ faecal *E. coli*. Trays were incubated at 37 ℃ for 22 to 24 hours before the Most Probable Number (MPN) of total coliforms and *E. coli* was determined.

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

Primer	**Specificity**	**Sequence 5'-3'**
UidAa (30 pmol)	*E. coli* O157:H7	GCGAAAACTGTGGAATTGGG
UidAb (30 pmol)	*E. coli* O157:H7	CGCTTTTGACACCTTAACCC
LMFP (20 pmol)	*L. monocytogenes*	AGCTCTTAGCTCCATGAGTT
LMRP (20 pmol)	*L. monocytogenes*	TCGAGAATCGAGGTACTCAA
SLDF (50 pmol)	*S.* Typhimurium	CCTGTGAATGCCCTGATGAT
SLDR (50 pmol)	*S.* Typhimurium	GGACACTTACGGGACTACTA
SCN2F (30 pmol)	*S. aureus*	TTGCATATGTATGGCAATTGTT
SCN2R (30 pmol)	*S. aureus*	AACGTATACATACCGTTAACAA

3.3.3 Pesticide analysis

- *Preparation of bacterial reference cultures*

The reference cultures used in this study were all obtained from the American Type Culture Collection (ATCC), Manassas, USA. The strains included *Escherichia coli* O157:H7 (ATCC 35150), *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028), *Listeria monocytogenes* serotype 4b (ATCC 19155) and *Staphylococcus aureus* subsp. *aureus* (ATCC 12600). Cultures were maintained at - 70 ℃ and subcultures were used for all subsequent tests.

Reference cultures were streaked out onto relevant selective media and incubated for 24 hours at 37℃. One hundred millilitres of tryptone soy broth (Merck, Johannesburg, South Africa) was inoculated with one colony and incubated in a shaking incubator (150 rpm) at 37℃. Following 18 hours incubation, a bacterial population of approximately 10^8 colony-forming units

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(cfu)/ml was achieved. The culture was then diluted to 10^4 cfu/ml, washed twice with sterile water and resuspention in 1 ml 1% (w/v) buffered peptone water for further experimental use.

A total of 20 pesticide products registered and commonly used for spraying of table grapes in South Africa were collected from the suppliers for analysis. These pesticides included insecticides and fungicides (Table 3.2), and all were diluted according to the manufacturer's recommendations using sterile water.

Table 3.2: Pesticide products tested, active ingredients and dilution concentrations

Trade name	Chemical group	Active ingredients	Active ingredient formulation	Spray dilution
Fungicides				
Sulphur	Inorganic	Sulphur	800 g/L	300 g/100L
Kumules	Inorganic	Sulphur	800 g/L	300 g/100L
Dithane	Dithiocarbomates	Mancozeb	800 g/L	150 g/100L
Copper hydroxide	Inorganic	Cupric hydroxide	538 g/kg	175 g/100L
Strobin	Strobilurin	Azoxystrobin	500 g/kg	15 g/100L
Capatan Flo	Phthalimide	Captan	500 g/L	150 ml/100L
Teldor 500 SC	Anilide	Fenhexamid	500 g/L	75 ml/100L
Rovral aquaflow	Dicarboximides	Iprodione	500 g/L	100 ml/100L
Prosper 500 EC	Unclassified	Spiroxamine	500 g/L	60 ml/100L
Brilliant SL	Inorganic	Ammonium phosphate	386 g/L	400 ml/100L
Indar	Triazoles/Conazole	Fenbuconazole	50/ g/L	80 ml/100L
Flint 50 WG	Strobilurin	Trifloxystrobin	500 g/kg	10 g/100L
Insecticides				
Dipel DF	Microbial	*Bacillus thuringiensis*	540 g/kg	15 g/100L
Azinophosmethyl	Benzotriazine	Azinphos methyl WP	350 g/kg	50 g/100L
Cypermethrin	Pyrethiods	Cypermerthrin EC	200 g/L	5 ml/100L
Bulldock	Pyrethiods	Betacyfluthrin	50 g/L	5 ml/100L
Chloropirifos	organophosphates	Chloropirifos	480 g/L	100 ml/100L
Erador	Botanical	Azadirachtin/ Pyrethrins	5.44 g/L	100 ml/100L
Acarol 500 EC	Acaricides	Bromopropylate	500 g/L	50 ml/100L
Thioflo	Organochlorines	Endosulfan	475 g/L	100 ml/100L

Nine millilitre of each pesticide solution was mixed with 1 ml of 10^4 cfu/ml culture and incubated at room temperature (20-22 ℃). For negative control, the same was done using sterile water with no pesticide product added. Following the relevant incubation period, 1 ml of the samples were taken after 0, 1, 4 and 8 hours and a dilution series was made with subsequent plating onto selective media [MacConkey with CV , XLD, Baird-Parker and Oxford-

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Listeria selective- agar (Merk)] in duplicate. Plates were incubated for 24 hours at 37℃ before colony counts were done to determine the cfu/ml.

3.3.4 Statistical analysis

All experiments were repeated twice and for each water sample, treatments were replicated five times. Mean populations of microbial load were subjected to analysis of variance using SAS 9.2 for windows (SAS Institute Inc., Cary, NC). The least significant difference ($P < 0.05$) test was used and expressed as log CFU between treatments.

3.4 Results

3.4.1 Water analysis

The average mean of data collected from two distinct seasons showed that dam water had significant variations in yeast growth, i.e. log 4.53 cfu/ml early in the season and log 2.84 cfu/ml from mid-season sampling. However, no significant count could be recorded for bacterial and fungal colonies (Table 3.3). Both river and spray tank sources showed no record of significant variation in yeast and fungal counts isolated during early and mid-seasonal treatments (Table 3.3). On the other hand, river water had significant higher log 5.94 cfu/ml of bacteria isolated on early-seasonal treatments when compared to log 5.02 cfu/ml of mid-season treatments. Early in the season, spray tank water revealed significantly higher bacterial counts of log 6.26 cfu/ml compared to log 5.52 cfu/ml of mid-seasonal counts. No microbes were recorded in spray solutions (Dithane and Teldor) used early in the season, while the spray solution applied during mid-season had higher counts of log 7.84 cfu/ml bacteria, log 6.61 cfu/ml yeasts and log 2.21 cfu/ml fungi.

The (MPN) of Colilert 18®-test showed that there was a correlation of coliform counts between water sources i.e. dam, river and spray tank water solutions (Table 3.3). The tests indicated that there was no major difference in *E. coli* counts between the early season and mid season treatment.

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Table 3.3: Comparison of total viable counts between early- and mid-seasonal sampling of water sources

Water source	Log (x+1) cfu/ml			MPN	
	Bacteria	Yeast	Fungi	Coliforms	E. coli
Dam	6.05 a	4.53 a	3.96 a	2203 a	1044.6 a
	6.31 a	2.85 b	2.92 a	2419.6 a	9.9 b
River	5.94 a	4.05 a	3.95 a	2221 a	659 a
	5.02 b	3.34 a	3.13 a	2177.3 a	50.2 a
Spray point	6.26 a	4.14 a	3.98 a	2376.3 a	1170.7 a
	5.52 b	2.78 a	3.25 a	2221 a	109.7 b
Spray solution	*0 b	*0 b	*0 b	*0 b	*0 b
	**7.84 a	**6.61 a	**2.21 a	**2420 a	**1351 a

Within the same water source, mean values that are not followed by the same letter are significant different ($P < 0.05$). *Spray solution- Dithane + Teldor 500 SC; **Spray- Teldor 500 SC

The study further confirmed a significant variation of the MPN of *E. coli* within the dam source and spray tank solutions. In both water sources, high MPN of *E. coli* were found in early-seasonal sampling with 1044.6 MPN of dam and 1170.7 MPN of spray tank water solutions compared to mid-seasonal samples with the least 9.9 MPN found in dam and 109.7 MPN in spray tanks. In the spray solutions, both coliforms and *E. coli* observed from early-seasonal were < 1MPN and the mid-seasonal solutions with significantly higher value of 2420 MPN coliforms and 1351.7 MPN of *E. coli*.

The microbial count study showed that water treatments (dam water, river water, spray tank water and spray solution) carried high bacterial, yeast and fungal loads (Table 3.4). Culturable total yeast counts ranged between log 3.30 cfu/ml in spray solutios, log 3.46 cfu/ml in water collected from the spray tank, log 3.69 cfu/ml in dam water and log 3.70 cfu/ml in river water.

Dam, River and spray tank water had similar fungal and bacterial loads. However, spray solutions recorded significant lower counts of log 1.10/ml cfu of fungi and log 3.92 cfu/ml of bacteria in relation to bacterial and fungal counts from dam, river and spray tank water sources.

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Table 3.4: Comparism of viable count between water treatments: dam water, river, spray point and spray solution.

Water	Log (x+1) cfu/ml			MPN	
Sources	Bacteria	Yeast	Fungi	Coliforms	E. coli
Dam	6.18 a	3.69 a	3.44 a	2311.3 a	527.3 a
River	5.51 b	3.70 a	3.31 a	2199.1 a	506.8 a
Spray point	5.89 ab	3.46 a	3.61 a	2298.6 a	640.2 a
Spray Solution	3.92 c	3.30 a	1.10 b	1209.8 b	675.9 a

*Mean values in the same column that are not followed by the same letter are significant different ($P<0.05$).

Two seasonal studies showed that high MPN of coliforms and *E. coli* were isolated from dam, river, spay tank and spray solutions (Table 3.4). Results showed that coliforms reached the maximum of 675.9 MPN and minimum MPN of 506.8; with the maximum 2311.3 MPN and the lowest 1209.8 MPN for *E. coli*. The average MPN value of coliforms statistically indicated a correlation between water collected from different sources i.e. dam, river, spray tank and spray solutions. The Colilert 18®-test further indicated that dam, river and spray treatment were highly contaminated with *E. coli* when compared to spray solution treatments. However, PCR confirmed that water sources were not contaminated with *E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium and *S. aureus* since none of these pathogens were found.

3.4.2 Pesticide analysis

Of the 20 pesticides tested, all allowed the survival of either one of the pathogens, except for Copper hydroxide (Table 3.5). After four to eight hours of mixing pathogen and pesticides, no viable count of *E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium in Dithane, Captan flo and Chloropirifos. No S. Typhimurium found in Chloropirifos immediately after inoculation. Dithane, Captan flo and Chloropirifos allowed survival of *S. aureus*, although *S. aureus* poorly survived in Dithane and Chloropirifos.

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Table 3.5: Pesticide products and a summary of results

	Active ingredients	***E. coli* 0157:H7**	***S. aureus***	***L. monocytogenes***	***S.* Typhimurium**
Sulphur	Sulphur	S	D_0	D_0	S
Kumules	Sulphur	S	D_0	D_0	S
Dithane	Mancozeb	D_8	S_D	D_4	D_4
Copperhydroxide	Cupric-hydroxide	D_4	D_4	D_4	D_4
Strobin	Azoxystrobin	G	S	S	S
Captan flo	Captan	D_8	S	D_8	D_8
Teldor 500 EC	Fenhexamid	S	S	G	S
Rovral aquaflow	Iprodione	S	S	S	S
Prosper 500 EC	Spiroxamine	S_D	S	D_0	D_0
Brilliant SL	Ammoniumphosphate	G	G	G	S
Indar	Fenbuconazole	S	S	D_1	D_4
Flint 50 WG	Trifloxystrobin	S	S	G	S
Dipel DF	*Bacillus thurringiensis*	S	S	S	G
Azinphos methyl	Azinphosmethyl WP	S	S	S	S
Cypermerthrin	Cypermerthrin EC	S	S	S	S
Bulldock	Betacyfluthrin	S	S	S_D	S
Chloropirifos	Chloropirifos	D_4	S_D	D_8	D_0
Erador	Azadirachtin/Pyrethrins	S	G	G	S
Acarol 500 EC	Bromopropylate	S_D	S	D_8	S
Thioflo	Endosulfan	S	S	S	S

D_0–No viable count found immediately after inoculation; D_1– No viable count found after one hour incubation in pesticide solution; D_4– No viable count found after four hours incubation in pesticide solution; D_8– No viable count found after eight hours incubation in pesticide solution; S_D–pathogen poorly survived or the number pathogen decrease with an increase in time; S–pathogen survived in pesticides solution; G–number pathogen increased in pesticide solution.

Inorganic pesticide products Sulphur and Kumules showed bactericidal effects on *S. aureus* and *L. monocytogenes* immediately after contact, while both permitted the survival of *E. coli* O157:H7 and *S.* Typhimurium (Table 3.5). Prosper 500 EC inhibited the viability of *L. monocytogenes* and *S.* Typhimurium instantly after contact. In an Indar product, there were no viable counts of *L. monocytogenes* between zero and one hour, and *S.* Typhimurium between one and four h after inoculation. *Escherichia coli* O157:H7 and *S. aureus* survived in Prosper 500 EC and Indar. *Escherichia coli* O157:H7 poorly survived in Prosper 500 EC.

Acarol 500 EC only had inhibitory effects on *L. monocytogenes* since there were no counts after four to eight hours. Acarol 500 EC therefore allowed the survival of *E. coli* O157:H7, *S. aureus* and *S.* Typhimurium, except that *E. coli*

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O157:H7 poorly survived. Pesticides products such as Rovral aquaflow, Azinphosmethyl, Cypermethrin, Bulldock and Thioflo permitted the survival of all pathogenic bacteria studied for the entire 8 hours incubation. However, the colony counts of *L. monocytogenes* significantly decreased in Bulldock solutions with an increase of incubation period.

The products- Strobin, Teldor 500 EC, Flint 50 WG and Dipel DF allowed multiplication of at least one of the pathogen studied. Teldor 500 EC and Flint 50 WG led to the multiplication and growth of *L. monocytogenes*, while *E. coli* O157:H7, *S. aureus* and *S.* Typhimurium survived in Teldor 500 EC and Flint 50 WG solutions. Strobin solutions favoured multiplication and growth of *E. coli* O157: H7, while *S aureus, L. monocytogenes* and *S*. Typhimurium survived thoughout the entire incubation period. Dipel DF allowed the survival of *E. coli* O157: H7, *S aureus* and *L. monocytogenes* and the increase of *S*. Typhimurium counts, and. Erador solutions maintained *E. coli* O157: H7 and *S.* Typhimurium at initial population levels. However, *S aureus* and *L. monocytogenes* multiplied in Erador solutions. Brilliant SL solution resulted in the multiplication of three pathogens studied - *E. coli* O157: H7, *S aureus* and *L. monocytogenes* and the survival of only *S*. Typhimurium.

In general, the study showed that *L. monocytogenes* was inhibited (no viable counts) in nine, *S* Typhimurium in six, *E. coli* O157:H7 in four and *S. aureus* in three pesticides tested. *Staphylococcus aureus* survived well in fifteen, *E. coli* O157:H7 in fourteen, *S.* Typhymurium in thirteen, *L. monocytogenes* in seven in pesticides tested. It was further found that *L. Monocutogenes* counts increase in four pesticide products, while *E. coli* O157:H7 and *S. aureus* showed the same in two pesticide products. *Salmonella* Typhimurium was only able to multiply in one pesticide product.

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3.5 Discussion

In this study, on farm sources of agricultural water were found to contain high faecal coliform and faecal *E. coli* counts. According to the South African National Standards of drinking water, no *E. coli* should be detected, while MPN of faecal coliform should not exceed 10 cfu/100ml of water samples. Both trails showed that there were high levels of microbial counts in water sources (dam, river and spray point).

Water analysis confirmed that microbes were largely dependent on the kind of pesticide product used i.e. no microbial count were recorded in a mixture of Dithane and Teldor spray while, Teldor spray alone allowed microbial multiplication. The *in vitro* study showed that Dithane was able to inhibit the survival of all foodborne pathogens studied, while Teldor 500 EC allowed the multiplication of *L. monocytogens* and survival of other foodborne pathogens. As such, Dithane have inhibitory characteristics on a wide range of microorganisms, with Teldor 500 EC having no inhibitory effects. Previous studies by Guan *et al.* (2001) and Ng *et al*. (2005), also demonstrated that the growth of foodborne pathogens such as *E. coli, L. monocytogenes* and *Salmonella* was inhibited in Dithane spray solution. However, their study also showed the survival and multiplication of these foodborne pathogens in other spray solutions. The effects of these insecticides on foodborne pathogen survival differed, except for the Lorsban 4E and Chlopirifos with the same active ingredients. In addition, Bravo 500 (chlorothalonil) from the previous study (Guan *et al*., 2001) and Captan flo (captan) falling within the phthalimide group also differed in terms of their effects on foodborne pathogens survival. However, results from this study showed that cypermerthrin (cypermerthrin EC) and Bulldock (betacyfluthrin) respectively systemic pyrethroids permitted survival of all pathogens studied. Similar results were also found with Strobin (azoxystrobin) and Flint 50 WG (trifloxystrobin) strobilurin fungicides.

Death, survival and multiplication of foodborne pathogenic bacteria (*E. coli* 0157:H7, *S. aureus*, *L. monocytogenes* and *S*. Typhimurium) in pesticide

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solutions is not dependent on the chemical groups but rather on particular product, temperature, pH, active ingredients, additives and adjuvants (Guan, 2005). The current study showed that the products Sulphur and Kumules with sulphur as active ingredient, showed similar characteristics of killing *S. aureus* and *L. monocytogenes*, but allowed survival of *E. coli* 0157:H7 and *S.* Typhimurium. It was reported by Ng *et al*. (2005), that Kumules DF with the same active ingredient as Kumules and Sulphur caused death of *L. monocytogenes* and permitted the survival of *E. coli* O157:H7 and encouraged the growth of *S.* Typhimurium. Previous studies also demonstrated that Penncozeb 750 DF with the same active ingredients as Dithane, and Champ Dry Prill having the same active ingredient as Copper hydroxide, were all able to inhibit the survival of *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium after different time intervals (Ng et al., 2005). Furthermore, Guan *et al.* (2001) demonstrated that Dithane M45 (Mancozeb) and Lorsban 4E (Chloropirifos) caused the death of *E. coli, L. monocytogenes* and *Salmonella* after one hour of contact and this correlates with the results observed in this study.

However, Guan *et al.* (2001) demonstrated that the product Ambush (permethrin) allowed survival and growth of *E. coli* 0157:H7, *L. monocytogenes* and *S.* Typhimurium and Ng *et al*. (2005) indicated that the same product with the same active ingredient inhibited the same bacteria. Variation in results reported in these studies may be attributed, because the used pesticide products were obtained from two different manufacturers. It was further reported that after inoculation, pesticide solutions were incubated at different temperatures. Therefore the ability of Ambush to kill and support foodborne pathogen was attributed to possible differences in additives and/or adjuvants present in the defferent pesticide formulations. The exact composition of pesticides, which includes adjuvants and inerts, is ussually not disclosed on the product label.

In this study, *Listeria monocytogenes* could not survive in most solutions tested whilst *E. coli* O157:H7, *S. aureus* and *S.* Typhimurium were able to

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survive in most of the product tested. Furthermore, Dithane, Copper hydroxide, Captan flo, Prosper 500 EC and Chloropirifos showed a negative growth effect against most of the pathogens tested. Water analysis tested negative for the pathogens investigated in this study i.e. *Escherichia coli* 0157:H7, *Listeria monocytogenes, Salmonella* Typhimurium and *Staphylococcus aureus*. Depending on the product, the use of highly contaminated agricultural water may to mix pesticides may lead to an increase and inhibition of microbes in the pesticide solution. It is therefore important for growers to regularly monitor water quality for compliance to regulatory requirements. Portable water should be used to prepare pesticide sprays. Application of product solutions immediately after preparation and/or using chemical spray products that inhibit pathogen growth is encouraged. Mixing two or more pesticide products in one spray tank is also ideal as their effects on foodborne pathogens differs.

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3.6 References

Brandl, M.R., 2006. Fitness of human enteric pathogens on plants and implications for food safety. *The Annual Review of Phytopathology* 44: 367-392.

Buck, J.W., Walcott, R.R. & Beuchat, L.R., 2003. Recent trends in microbiological safety of fruits and vegetables. Plant health progress. www.cspinet.org/foodsafety/outbreak-alert.pdf. Accessed: 3 March 2008.

Buzby, J.C., Roberts, T., Jordan Lin, C.T. & MacDonald, J.M., 1996. Bacterial foodborne disease: Medical costs and productivity losses. Food and consumer economics division, economic research service, U.S. Department of Agriculture. Agricultural economic report no. 741. www.ers.usda.gov/Publications/AER741/. Accessed: 3 March 2008.

Coghlan, A.C., 2000. Food poisoning bugs in crop spray. *New Scientist*: 20.

De Roever, C., 1998. Microbiological safety evaluations and recommendations on fresh produce. *Food Control* 9: 321-347.

De Waal, C.S., Johnson, K. & Bhuiya, F., 2007. Outbreak alert! 2006. Center for science in the public interest. www.cspinet.org/foodsafety/outbreak-alert.pdf. Accessed: 3 March 2008.

Food and Beverage Report, 2008. Health, nutrition, hygiene. Curbing contamination. p. 32-35.

Guan, T.Y. Bank, G., Ismond, A. & Van Acker, R., 2001. Fate of foodborne bacterial pathogens in pesticide products. *Journal of the Science of Food and Agriculture* 81: 503-512.

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Guan, T.Y., Blank, G. & Holley R.A., 2005. Survival of pathogenic bacteria in pesticides solutions and on treated tomato plants. *Journal of Food Protection* 68: 296-304.

Izumi, H., Tsukada, Y., Poubol, J. & Hisa, K., 2008. On-farm sources of microbial contamination of persimmon fruit in Japan. *Journal of Food Protection* 71: 52-59.

Kirby, R.M., Bartram, J. & Carr, R., 2003. Water in food production and processing: quantity and quality concerns. *Food Control* 14: 283-299.

Koutsotoli, A.D. & Dimou, D.S., 2005. Long term and short term inducible effects of trifluralin on *Escherichia coli* and *Enterococcus faecalis. Microbial Ecology in Health and Disease* 17: 121-127.

McEwen, F.L., 1997. Pesticide management and insecticide resistance. In: Pesticides residue and agriculture workers – An overview. page 37. In: D.L. Watson & A.W.A. Brown (Eds). Academic Press, New York, USA.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. & Tauxe, R.V., 1999. Food-related illness and death in the United States. *Emerging Infectious Diseases* 5: 607-625.

Michino, H., Araki, K., Minami, S., Takaya, S., Sakai, N., Miyazaki, M., Ono, A. & Yanagawa, H., 1999. Massive outbreak of *Escherichia coli* 0157:H7 infection in schoolchildren in Sakai city, Japan, associated with consumption of white radish sprouts. *American Journal of Epidemiology* 150: 787-796.

Ng, J.P., Graham, H.F. & Gillian, M.H., 2005. Pesticides as a source of microbial contamination of salad vegetables. *International Journal of Food Microbiology* 101: 237-250.

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Nystrom, B., Bjornsater, B. & Blanck, H., 1999. Effects sulfonylurea herbicides on non-target aquatic microorganisms growth inhibition of micro algae and short term inhibition of adenine and thymidine incorporation in periphyton communities. *Aquatic Toxicology* 47: 9-22.

Trench, T.N., Wilkinson, D.J. & Esterhuysen, S.P., 1992. Pesticides formulation. Pages 111-114. In: South African plant disease handbook: Farmer Support Group. Rev: McIntyre, J., Bayer, Pietrmartzburg, SA.

Wang, H. & Slavik, M.F., 2005. A multitex polymerase chain reaction assay for rapid detection *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella* Typhimurium and *Campylobacter jejuni* in artificially contaminated food samples. *Journal of Rapid Methods and Automation in Microbiology* 13: 213-223

Ware, G.W., 1986. Pesticides chemistry. Pages 13-20. In: Revised Fundamentals of pesticides. A self instruction guide. 2nd Edition. Thomson publications, USA.

World Health Organisation, 2005. The WHO recommended classification of pesticides by hazard and guideline to classification: 2004 www.who.int/ipcs/publications/pesticides_hazard_rev_3. Accessed: 3 March 2009.

Zhao, T., Doyle, M.P. & Besser, R.E., 1993. Fate of Enterohemorrhagic *Escherichia coli* 0157:H7 in Apple cider with and without preservatives. *Applied and Environmental Microbiology* 59: 2526-2530.

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Chapter 4: Evaluation of microbial quality of table grapes, and attachment, colonization and survival potential of *Escherichia coli* 157:H7, *Listeria monocytogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* on table grape surfaces

4.1 Abstract

Table grapes are an important export crop in South Africa and must comply with international food safety standards. Table grapes were collected directly from the vineyard and analysed to determine the microbial population dynamics and possible presence of certain foodborne pathogens. Presumptive *Escherichia coli*, *Listeria* spp, and *Staphylococcus* spp were isolated from field harvestion table grapes. However, no positive confirmation could be made of the pathogenic strains isolated using PCR analysis. Under artificial post-harvest simulated conditions, the attachment and survival of *E. coli* O157:H7, *L. monocytogenes, S.* Typhimurium and *S. aureus* were studied. The presence of pathogens was determined by plate count and scanning electron microscopy (SEM). Berries were inoculated individually with 10^5 cfu/ml of each of the prepared four pathogens. Viable counts were done immediately and after 30's, 15 min, 1hour, 7days and 9days. *Staphylococcus aureus* and *E. coli* O157:H7 survived, followed by *S.* Typhimurium under these tested storage conditions. *Listeria Monocytogenes* was least able to survive under these conditions. However, SEM counts after 7d at - 2 ℃ were respectively higher than *E. coli* O157:H7, *S.* Typhimurium and *S. aureus* viable counts. Adhesion of all pathogens was visually observed using the SEM immediately after exposure. *Listeria monocytogenes, S.* Typhimurium and *S. aureus* cells colonised the table grape berry surfaces uniformally within 15 min to 1 hour. The study showed the potential of foodborne pathogens to attach, colonize and survive on table grape surfaces under laboratory conditions.

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4.2. Introduction

Consumption of raw or minimally processed fresh produce (fruit and vegetable), has increased dramatically due to their convenience and healthier dietary importance (Lin & Wei, 1997; Johannessen *et al*., 2002; Ilic *et al*., 2008). However, the increased consumption of fresh produce has also been associated with increased outbreaks of foodborne diseases (De Roever, 1998). Fresh produce related illness were also found to be favored by certain production practices, processing and preservation methods, packaging materials and distribution system (Beuchat, 2002; Ng et al., 2005; Izumi et al., 2008).

Foodborne pathogens may contaminate fresh produce at any stage during the production cycle (De Roever, 1998). Potential sources of contamination includes soil, faeces, irrigation water, water used to apply pesticides, sewage sludge, dust, insects, manure, wild and domestic animals, human handling, harvesting equipment, transport vehicles, rinse water and processing equipment (Beuchat, 2002; Johannessen *et al*., 2002; Canadian Horticultural Council. 2006). The numerous sources of potential contamination makes the prevention of the spread of foodborne pathogens a challenge for any production system (De Roever, 1998). The principal exposure of waterborne pathogens to humans is through ingestion of contaminated drinking water or contaminated food and hand-to-mouth remission; personal contact, droplet transfer, dermal contact or inhalation of contaminated aerosols (Grabow, 1996; Craun *et al*., 2006).

Foodborne pathogens have also been linked with disease outbreaks (De Roever, 1998) through consumption of several vegetables i.e. lettuce, cabbage, carrots, red pepper, cucumber and tomatoes, fruit i.e. apples, oranges, strawberries, mangoes) (Yu *et al*., 2001; Hean & Jones, 2007) and other processed products (fruit juices and salads) (Cook *et al*., 1998). Several studies have shown that ready to eat fruit and vegetables can harbour or support the growth of *Salmonella* spp. (Wei *et al*., 1995; Izumi *et al*., 2008),

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enteric bacterial pathogens such as *Shigella* spp. (Escartin *et al*., 1989) and *Escherichia coli* O157:H7 (Abdul-Raouf *et al*., 1993), *Listeria monocytogenes* (Beuchat, 1996) and *Staphylococcus aureus* (Thunberg *et al*., 2002). *Listeria monocytogenes* has been demonstrated not to survive on tomatoes and carrots (Heisick *et al*., 1989; Nguyen-The & Lund, 1991). In contrast, *L. monocytogenes*, *E. coli* O157:H7 and *S.* Typhimurium showed the ability to rapidly attach and colonise on lettuce and cabbage surfaces (Takeuchi *et al*., 2000; Solomon *et al*., 2002; Ells & Hansen, 2006). On the other hand, *Escherichia coli* O157:H7 was reported to grow on apple tissue and the growth was linked to the ability of the bacteria to modify the micro-environment on the apple surface (Dingman, 1999). For example, *E. coli* O157:H7 (Buchanan & Edelson, 1996; Ryu *et al*., 1999) and *Salmonella* spp. (Zhuang *et al*., 1995; Cook *et al*., 1998) grew at reduced pH levels that is not known to allow the survival of these foodborne pathogens. In most food products, temperatures between 20-30 ℃ are considered favorable for survival and growth of pathogens such as *E. coli* (De Roever, 1998), *S. aureus* (Spasford *et al*., 2004), *Salmonella* spp. (Harris *et al*., 2003). However, *L. monocytogenes* can survive and proliferate at both low (< 1 ℃) and room (20 – 25 ℃) temperatures (Watkins & Sleath, 1981). The capability of waterborne pathogens to attach or grow on fruit surface is influenced by the ability of these organisms to adapt to ecological factors and the physical environment of fresh produce (Beuchat, 2002; WHO, 2002).

Like any other fresh produce, table grapes only undergo minimal processing and have not been associated with the transmission of foodborne pathogens to humans. Therefore, this study focused on the possibility that a suitable environment and surface for foodborne colonization may exist on table grapes surfaces. The aim of this study was to determine the microbiological load and presence of *Escherichia coli* O157:H7, *Salmonella enterica* subsp. *enterica* serotype Typhimurium, *Listeria monocytogenes* and *Staphylococcus aureus* on table grapes surfaces. The study further assess the ability of *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes* and *S. aureus* to attach, colonise, survive and replicate on the fructoplane.

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4.3 Materials and methods

- *Preparations of bacterial references cultures*

Reference cultures used in this study were handled and prepared as described in Chapter 3, section 3.3.3.

4.3.1 Table grape microbiological analysis

The study was carried out over two growing seasons (2008/2009 and 2009/2010). Fruit berries (Saltana) collected from one producing farm in the Western Cape. From each growing season, trials were done early-, mid- and late in the season collected from the vineyard. During the field trials, fruit (5 x 1kg) was collected before and after the application of the commercial fungicides spray solution. In each growing season, one trial was done by collecting grapes from the packhouse after packing.

Five batches of one–kilogram replicate of fruit were analysed from each of the five sampling points. Harvested fruit was placed in a labeled bag and then in a box for transport purposes to the laboratory. Fruit berries were aseptically cut from the stems randomly, weighed and sonicated in 500 ml Ringer's solution containing 0.02% (v/v) Tween 80 (Merck). Each washing solution was filtrated through cellulose nitrate filters, pore size 0.45 µm (Sartorius, Goettingen, Germany). Each filter paper was transferred to 9 ml of TSB, vortexed and one millilitre of TSB solution was transferred to a 9 ml of Ringer's solution for a dilution series. Solutions were plated out in duplicate onto selective media (MacConkey, Oxford-Listeria selective agar, Baird-Parker and XLD), Standard 1 agar (STD 1) and Malt Extract agar (MEA). Selective media were incubated for 24 hour at 37 ℃, while STD 1 and MEA plates were incubated for 7 days at 25 ℃ before colony count. During early- , mid- and late seasonal samplings, microbial counts as determined from fruit before and after spraying were compared.

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The TSB solution of washed fruit samples were incubated in a shake incubator for 48 hours at 37 ℃ before a DNA extraction was done using a 1% Triton X-100 DNA extraction protocol. Presence of presumptive organisms were identified and confirmed using a multiplex Polymerase Chain Reaction (PCR) method as described in Chapter 3, section 3.3.2.

4.3.2 Artificial inoculation studies

Table grapes (Saltana) were collected and transported to the laboratory (University of Pretoria) and analysed within 24 hours. Grape berries were surface sterilised using a 70 % ethanol dip and air dried for SEM studies and spiking trail fruit were dipped into 9 % of sodium hypochlorite bleach and dried; for 30 s respectively.

A 50 µl of each pathogens prepared solution (Chapter 3, section 3.3.3) (10^5 cfu/ml) were placed on the periphery of the fruit surface and after each specific time interval (immediately, 30 s, one min, 15 min, one hour, seven days and nine days), the liquid was removed from the surface using a pipette and washed twice with sdH_2O by depositing and removal.

For both spiking and SEM trials, fruit have been evaluated immediately and 1h after inoculation were left at 20-22 ℃ and those analysed after 7d were kept at 0 to -2 ℃ for the duration of the experiment to simulate the commercial export cold chain. Fruit evaluated after 9d were kept at 0 to -2 ℃ for the first seven days and then left at 20-22 ℃ for the last two days before further processing.

4.3.2.1 Scanning electron microscopy

Following the described intervals, inoculated blocks were cut in preparation of SEM trial. Blocks were subsequently rinsed three times in 15 min intervals with 50 % of 1.5 M Phosphate buffer. Following the 15 min intervals, the blocks were dipped three times in 50 % (v/v), 70 % (v/v), 90 % (v/v) and 100

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% (v/v) ethanol; and the blocks were left in 100 % (v/v) ethanol solution, rinsed three times before dehydration. After dehydration, samples were mounted on a metal slide and overlayed with a thin layer of gold metal in a coating machine (Polaron equipment Ltd, England). Once plated, 30 spots of each sample were viewed at X 4000 magnification and the number of cells counted per viewing area.

4.3.2.2 Spiking fruit

After the described intervals (Section 4.3.2), 500 ml of Ringer's solution with 0.02 % Tween 80 was used to wash fruit. The solution was filtered through a 0.45 µm filter. All solutions were then plated out in duplicate onto MacConkey, Oxford-Listeria selective agar, Baird-Parker and XLD and plates were incubated for 24 hour at 37 ℃ before viable counts for presence or absence of the pathogens were determined.

4.3.3. Statistical analysis

Field and spiking experiments were repeated twice, and for each treatment five replicates were done. The SEM experiment was not repeated but was replicated three times. Mean populations of microbial load were subjected to analysis of variance using SAS 9.2 for windows (SAS Institute Inc., Cary, NC). The least significant different ($P < 0.05$) test was used to determine significant differences expressed as log CFU between treatments.

4.4 Results

4.4.1 Table grape microbiological analysis

Bacterial counts showed no significant difference on both chemical sprayed and unsprayed fruit with early- and mid seasonal samples (Table 4.1). Significant lower values of bacteria, yeast and fungi were recorded between late seasonal fruit and harvested fruit taken up in the packhouse.

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Table 4.1: Total viable counts between:- untreated and pesticide treated fruit early- and mid- season; and fruit collected late season and after harvest and those collected from packhouse on late season samples

Fruit samples	Log (x+1) cfu: Bacteria	Yeast	Fungi
Early season - Untreated	5.54 a	4.36 a	4.22 a
- Treated	*5.40 a	*4.35 a	*4.30 a
Mid- season - Untreated	5.88 a	5.33 a	4.08 a
-Treated	**5.72 a	**5.14 a	**4.05 a
Late- season - In orchard	5.75 a	5.69 a	4.20 a
- In packhouse	5.03 b	4.53 b	2.54 b

Within the same fruit samples, mean values that are not followed by the same letter are significant different ($P < 0.05$). Value obtained after the fruit were treated with - *Dithane + Teldor 500 SC; and -** Teldor 500 SC

The average microbial load on fruit were then compared throughout the growing season (early-, mid- and late season fruit) and it was found that bacteria and yeast were predominately isolated from fruit surfaces (Table 4.2).

Table 4.2: Comparism of viable plate counts of washed fruit berries collected: early-, mid- and late- seasonally

Ready to harvest fruit	Log (x+1) cfu: Bacteria	Yeast	Fungi
Early- seasonal	5.47 ab	4.36 b	4.26 a
Mid- seasonal	5.80 a	5.24 a	4.07 a
Late- seasonal	5.39 b	5.10 a	3.37 b

*Mean values in the same column that are not followed by the same letter are significant different ($P<0.05$).

Higher bacterial counts were recorded on mid- (log 5.80 cfu), compared to late- (log 5.38 cfu) season grapes, but early seasonal fruit did not have statistically less or more bacteria. Yeast increased significantly from mid seasonal fruit when compared to the early seasonal fruit. In contrast fungal loads were the lowest in late seasonal fruit.

4.4.2 Artificial inoculation studies

Initial adhesion: Bacterial attachment was evident with SEM studies immediately after exposure to the grape berry surface for all four pathogens tested (Table 4.3). *Staphylococcus aureus* attachment was only evident after

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1 min exposure compared to *E. coli* and *L. monocytogenes* that attached within 30 s. *Salmonella* Typhimurium attached immediately after exposed to a berry fruit.

Attachment: Foodborne pathogenic bacteria produced exopolysaccharide structures on grape berry surfaces. *Salmonella* Typhimurium showed attachment structures immediately after exposure to the grape fructoplane. Attachment structure for both *E. coli* O157:H7 and *L. monocytogenes* were observed on grape 30 s after inoculation. The exopolysaccharide structure of *S. aureus* was produced on grape after 1 min.

Colonisation: All four pathogens produced a biofilm as early as between 30 s and one min., but the formations extensively increased with extended incubation times. *Escherichia coli* O157:H7 immediately attached to grape berry surfaces and reached the highest number of cells after one hour, and were more notable around lenticels (Table 4.3). For *L. monocytogenes* cells, increased consistently on the surface with the highest counts being recorded after nine days. *Listeria monocytogenes* continued to replicate and invaded micro niches on the grape surfaces at storage temperature of - 2 ℃.

Table 4.3: Comparison of different time intervals required for *E. coli* O157: H7, *L. monocytogenes, S.* Typhimurium and *S. aureus* attachment and colonization to berry fruit surface as viewed under the scanning electron microscope

	Time recorded observing:	
Pathogens	**Attachment**	**Colonization**
E. coli O157: H7	30 s	60 min
L. monocytogenes	30 s	60 min
S. Typhimurium	0 s	15 min
S. aureus	60 s	15 min

This was further confirmed by high numbers of cells per cm^2 on grape skin surface (Table 4.4). *Salmonella* Typhimurium showed glycocalyx formation and colonization at 15 min after inoculation, and respectively reproduced on grape surface up to the end of simulated cold storage conditions. A significant

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increase in *Staphylococcus aureus* cells per cm^2 was notable after one min. (Table 4.4) and the extensive glycocalyx formation were initially found on the grape skin after 15 min of inoculation.

Table 4.4: Comparison of total human pathogenall counts on fructoplane evaluated through scanning electron microscopy from grape samples stored in the simulated table grape supply chain

	Log (x+1) cells/cm^2			
Time	*E. coli* O157: H7	*L. monocytogenes*	*S.* Typhimurium	*S. aureus*
0 s	5.98 b	5.66 e	5.07 d	5.53 c
30 s	5.55 c	5.85 de	5.21 d	5.59 c
1 min	5.67 bc	5.80 de	5.91 c	6.19 b
15 min	5.88 bc	5.96 dc	6.45 ab	6.55 a
1 hour	6.57 a	6.16 c	6.32 b	6.51 a
7 days	5.53 c	6.62 b	6.46 ab	6.59 a
9 days	5.53 c	7.06 a	6.56 a	6.74 a

*Mean values in the same column that are not followed by the same letter are significant different ($P<0.05$).

Survival of pathogen: Survival and behaviour of four foodborne pathogens on grape surface is summarised in table 4.5. In the study both temperature and period of incubation had a significant effect on *E. coli* O157:H7 and *S.* Typhimurium; and no significant effect were recorded on *L. monocytogenes* and *S. aureus*. Immediately after application, *E. coli* O157:H7 viable counts were lower than counts recorded after different time intervals. The -2 ℃ at incubated samples retained for seven days had a significant effect on *E. coli* O157:H7 and *S.* Typhimurium and led to a significant decrease in viable counts.*Escherichia coli* O157:H7 counts and were significantly related to immediately at 22 ℃ and 7 days at -2 ℃, under the same condition *S.* Typhimurium counts were also related. On the other hand, the incubation period of 30 s, one min, 15 min, 60 s and nine days showed no significant effects on *E. coli* O157:H7. Viable counts of *S.* Typhimurium were significantly lower at immediately inoculation and after 30 s. However, viable counts

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significantly increased between immediately after inoculation and 60 s at 22 ℃.

Table 4.5: Comparison of total human bacterial cells on spiked table grape surface evaluated after stored in the simulated table grape supply chain

	Log (x+1)			
Time	*E. coli* O157: H7	*L. monocytogenes*	*S.* Typhimurium	*S. aureus*
0 s	4.24 b	4.10 b	4.15 cd	5.18 ab
30 s	5.32 a	3.93 b	4.54 bc	5.55 ab
1 min	5.24 a	4.52 ab	4.99 ab	5.31 ab
15 min	5.39 a	4.69 ab	5.27 a	5.32 ab
1 hour	5.37 a	4.39 ab	5.36 a	6.31 a
7 days	3.90 b	4.54 ab	3.82 d	5.11 b
9 days	5.27 a	5.18 a	5.17 a	5.83 ab

*Mean values in the same column that are not followed by the same letter are significant different (P<0.05).

Salmonella Typhimurium cells were statistically related on the incubation period of 15 min, one hour and nine days. In general, the increase in incubation period was accompanied by the increase of *S.* Typhimurium cells. Both *L. monocytogenes* and *S. aureus* counts were independent from time and temperature. The observed *L. monocytogenes* inoculated onto grape surfaces were similar immediately, after 30 s, 60 s, 15 min, one hour and seven days incubation, but viable counts after nine days inoculation were significantly different from counts observed immediately and after 30 s. When viewed under the scanning electron microscope, *L. monocytogenes* cells were higher when stored at - 2 ℃ when compared to other three pathogens under study (Table 4.4). S*taphylococcus aureus* cells were notably different between one hour at 22 ℃ and seven days at -2 ℃, while there was a relation of *S. aureus* counts on other time intervals.

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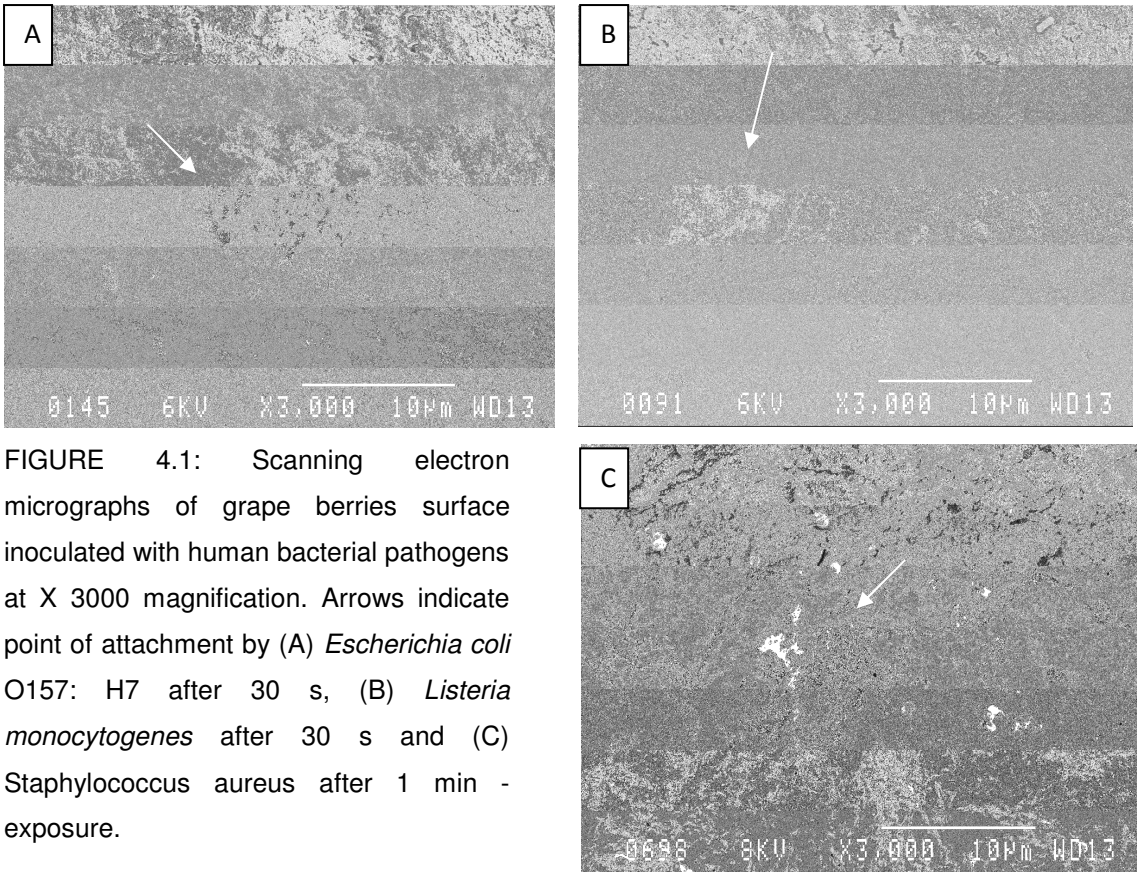

FIGURE 4.1: Scanning electron micrographs of grape berries surface inoculated with human bacterial pathogens at X 3000 magnification. Arrows indicate point of attachment by (A) *Escherichia coli* O157: H7 after 30 s, (B) *Listeria monocytogenes* after 30 s and (C) Staphylococcus aureus after 1 min - exposure.

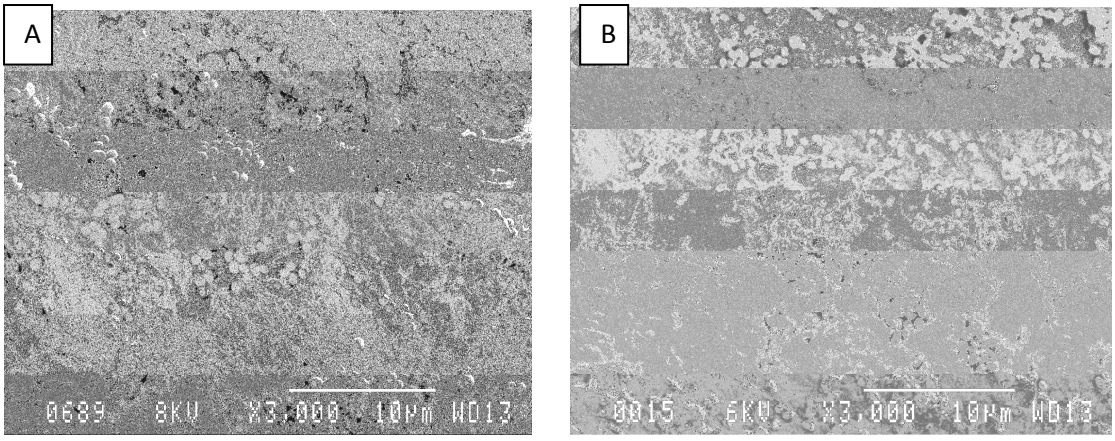

FIGURE 4.2: Scanning electron micrographs of grape berries surface inoculated with foodborne bacterial pathogens viewed at X 3000 magnification. Microcolony formation of: (A) *Staphylococcus aureus* after 15 min exposure, (B) *Listeria monocytogenes* nine days after inoculation.

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4.5. Discussion

The main findings of this study were that all foodborne pathogens tested showed the potential to attach, colonise and survive on grape skins. However, the patterns of colonisation greatly dependent on the type of pathogen evaluated and the incubation period. Previous studies also provide evidence that human pathogens can be detected and survive on plant tissue (Lang et al., 2004 Lapidot *et al*., 2006; Aruscavage *et al*., 2008). However, there is no direct evidence on the interaction and behaviour of these pathogens on grape surfaces.

Studies by Amoah et al. (2006) and Izumi et al. (2008) showed that contamination of fruit may occur in the field during the production process. Although there was no positive confirmation of foodborne pathogens, plate counts indicated that *E. coli, Staphylococcus* spp and *Listeria* spp were the most frequently isolated from fruit samples. No *Salmonella* spp. was isolated in this study. Viable counts of the isolated microorganisms were independent from the immediate applied spray solutions, but might have accumulated with time and continuous use of field spray throughout the season. This was confirmed by the results found during comparison of fruit before and after pesticide spray i.e. in both early- and mid-seasonal samples there was a no different between counts of chemical sprayed and unsprayed fruit. Though the level of microbial load decreased on fruit contained under cold storage conditions (- 2 ^{o}C), contamination that takes place during pre-harvest stage could have extend further to the post- harvest stage.

The *in vitro* study indicated that four pathogens adhered immediately after contact with grapes surface. *Salmonella* Typhimurium rapidly produced attachment structures immediately after being exposed to the grape surface, while *E. coli* O157:H7 and *L. monocytogenes* required 30 s, and *S. aureus* required one min to effectively attach in grape surfaces. This finding is in agreement with other studies, Collignon and Korsten (2010), showed the ability of *S. aureus*, *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium

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to adhere and attach to peach surface within 30 s to 60 s. In general, this study showed that *S.* Typhimurium adapts more rapidly, followed by *E. coli* O157:H7 and *L. monocytogenes*, and then *S. aureus.* Similarly, Beuchat and Scouten (2004), found that Salmonella spp. exhibited the strongest attachment on cantaloupe ring when compared to *E. coli* O157:H7, and *L. monocytogenes.* The adhesion and attachment mechanisms of all four pathogens were supported by the production of biofilm formation within the average incubation period of 60 s. The study by Lapidot *et al.* (2006), revealed that the biofilm matrix is a critical factor in the attachment and persistence of human pathogens to plant tissues.

In this study, time was found to be a critical factor in colonisation of grape surface. *Salmonella* Typhimurium and *S. aureus* colonised the grape surface within 15 min, while *E. coli* O157:H7 and *L. monocytogenes* were able to colonise and invade one hr after exposure to grape surface. Microscopic observations confirmed *E. coli* 0157: H7 cells near or around the lenticels of grape surfaces, while other foodborne pathogens were recorded in higher numbers in lesion and cracks of the surface. *Staphylococcus aureus* and *S.* Typhimurium effectively colonized and invaded grape berry (i.e. large micro-colony formations were observed 15 min after the pathogens were exposed to fruit surfaces). On the other hand, the largest micro-colony formation was found with *E. coli* O157: H7 and *L. monocytogenes*, which were observed on grape skin after seven days.

In this work, the extent and degree of attachment and colonization of *S.* Typhimurinm and *S. aureus* resulted in better survival and growth on grape surfaces. The inability of *E. coli* O157: H7 and *L. monocytogenes* to rapidly colonise the grape surface could have contributed to the less effective survival of these pathogens in the rest of the simulated supply chain. Of the four foodborne bacterial pathogens; *L. monocytoges, E. coli* O157:H7 and *S. aureus* showed the ability to survive on fruit surface at -2 ^{o}C, but *E. coli* O157:H7 and *S. aureus* were found not to grow well. This was in agreement with the study of Glesson and O'Beirne (2005), found a similar survival

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patterns for *Listeria* spp. and *E. coli* O157:H7 on both carrots and lettuce respectively. Though low counts were observed at some point in the supply chain, the study revealed that all foodborne bacterial pathogens under artificial high initial inoculation conditions remained higher than the minimum infectious dose.

The study indicated that *E. coli* O157:H7, *L. monocytogenes, S.* Typhimurium and *S. aureus* could possible attach, survive and colonize fruit skin, and their behavior is independent from time and temperature. It is however important to consider that this study was done when high artificial inoculation levels of these pathogens were used under simulated exposure conditions to grape berry.

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4.6. References

Abdul-Raouf, U.M., Beuchat, L.R. & Ammar, M.S., 1993. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. *Applied and Environmental Microbiology* 59: 1999-2006.

Amoah, P., Drechsel, P., Abaidoo, R.C. & Ntow, W.J., 2006. Pesticides and pathogen contamination of vegetables in Ghana's urban markets. *Archives of Environmental Contamination and Toxicology* 50: 1-6.

Anklam, E. & Battagilia, R., 2001. Food analysis and consumer protection. *Trends in Food Science and Technology* 12: 197-202.

Aruscavage, D., Miller, S.A., Ivey, M.L.L., Lee, K. & LeJeune, J.T., 2008. Survival and dissemination of *Escherichia coli* O157:H7 on physically and biologically damaged luttuce plants. *Journal of Food Protection* 71: 2384-2388.

Bastos, R.K.X. & Mara, D.D., 1995. The bacterial quality of salad crops drip and furrow irrigated with waste stabilazation pond effluent: an evaluation of the WHO guidelines. *Water Science and Technology* 31: 425-430.

Beuchat, L. R., 1996. *Listeria monocytogenes*: incidence on vegetables. *Food Control* 7: 223-228.

Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Journal of Microbes and Infection* 4: 413-423.

Beachat L. R. & Scouten, A. J., 2004. Factors affecting survival, growth and retrieval of *Salmonella poona* on intact and wounded cantaloupe rind and in stem scar tissue. *Food Microbiology* 21: 683-694.

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Buck, J.W., Walcott, R.R. & Beuchat, L.R., 2003. Recent trends in microbiological safety of fruits and vegetables. Plant health progress. www.cspinet.org/foodsafety/outbreak-alert.pdf. Accessed: 3 March 2008.

Buchanan, R.L. & Edelson, S.G., 1996. Culturing enterohemorrhagic *Escherichia coli* in the presence and absence of glucose as a simple means of evaluating the acid tolerance of stationary-phase cells. *Applied and Environmental Microbiology* 62: 4009-4013.

Canadian Horticultural Council, 2006. Leafy vegetables and cruciferae. On-farm food safety manual. Version 1 draft. Canadian Horticultural Council, Ottawa, Ontario, Canada. Accessed: 3 March 2008.

Collignon, S. & Korsten, L., 2010. 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. *Journal of Food Protection* 73: 1247-1256.

Conway, W.S., Leverentz, B. & Saftner, R. A., 2000. Survival and growth of *Listeria monocytogenes* on fresh-cut apple slices and Its Interaction with *Glomerella cingulata* and *Penicillium expansum. Plant Disease* 84:177-181.

Cook, K.A., Dobbs, T.E., Hlady, G., Wells, J.G., Barret, T.J. & Duhr, N.D., 1998. Outbreak of *Salmonella* serotype Hartfold infections associated with unpasturized orange juice. *Journal of American Medical Association* 280: 1504-1509.

Craun, G.F., Calderon, R.L. & Wade, T.J., 2006. Assessing waterborne risks: an introduction. *Journal of Water and Health* 4: 1-16.

Cummings, K., Barrett, E., Mohle-Boetani, J.C., Brooks, J.T., Farrar, J., Hunt, T., Foire, A., Komatsu, K., Werner, S.B. & Slutsker, L., 2001. A multistate

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outbreak of *Salmonella* enterica serotype bail-don associated with domestic raw tomatoes. *Emerging Infectious Diseases* 7: 1046-1048.

De Roever, C., 1998. Microbiological safety evaluations and recommendations on fresh produce. *Food Control* 9: 321-347.

De Waal, C.S., Johnson, K. & Bhuiya, F., 2007. Outbreak alert! 2006. Center for Science in the Public Interest. www.cspinet.org/foodsafety/outbreak-alert.pdf. Accessed: 3 March 2008.

Dingman, D.W., 1999. Growth of *Escherichia coli* O157:H7 in bruised apple (*Malus domestica*) tissue as influenced by cultivar, date of harvest, and source. *Applied and Environmental Microbiology* 66:1077-1083.

Ells, T.C. & Hansen, L.T., 2006. Strain and growth temperature influence *Listeria* spp. attachment to intact and cut cabbage. *International Journal of Food Microbiology* 111: 34-42.

Gandhi, M., Golding, S., Yaron, S. & Mathews, K.R., 2001. Use of green fluorescent protein expressing *Salmonella* Stanley to investigate survival, spatial location, and control on alfalfa sprouts. *Journal of Food Protection* 64: 1891-1898.

Gleesson, E. & O'Beirne, D., 2005. Effects of process severity on survival and growth of *Escherichia coli* and *Listeria innocua* on minimally processed vegetables. *Food Control* 16: 677-685.

Gorski, L., Palumbo, J.D. & Mandrell, R.E., 2003. Attachment of *Listeria monocytogenes* to radish tissue is dependent upon temperature and flagellar motility. *Applied and Environmental Microbiology* 69: 258-266.

© University of Pretoria

Guan, T.Y. Bank, G., Ismond, A. & Van Acker, R., 2001. Fate of foodborne bacterial pathogens in pesticide products. *Journal of the Science of Food and Agriculture* 81: 503-512.

Guan, T.Y., Blank, G. & Holley R.A., 2005. Survival of pathogenic bacteria in pesticides solutions and on treated tomato plants. *Journal of Food Protection* 68: 296-304.

Hamilton, P.D., Gale, P. & Pollard, S.J.T., 2006. A commentary on recent water safety initiatives in the context of water utility risk management. *Environment International* 32: 958-966.

Harris, L.J., Farber, N.J., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H. & Busta, F.F., 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh cut produce. *Comprehensive Reviews in Food Science and Food Safety* 2: 78-141.

Hedberg, C.W., Angulo, F.J., White, K.E., Langkop, C.W., Schell, W.L., Stobierski, M.G., Schuchat, A., Besser, J.M., Dietrich, S., Helsel, L., Griffin, P.M., Mcfarland, J.W. & Osterholm, M.T., 1999. Outbreak of Salmonellosis associated with eating uncooked tomatoes: Implications for public health. The investigation team. *Epidemiology and Infection* 122: 385-393.

Heisick, J.E., Wagner, D.E., Nierman, M.L. & Peeler, J.T., 1989. *Listeria* spp. found on fresh market produce. *Applied and Environmental Microbiology* 55: 1925-1927.

Ilic, S., Odomeru, J. & Lejeune, J.T., 2008. Coliforms and prevalence of *Escherichia coli* and foodborne pathogens on minimally processed spinach in two packing plants. *Journal of Food Protection* 71: 2398–2403.

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Izumi, H., Tsukada, Y., Poubol, J. & Hisa, K., 2008. On-farm sources of microbial contamination of persimmon fruit in Japan. *Journal of Food Protection* 71: 52-59.

Johannessen, G.S., Locarevic, S. & Kruse, H., 2002. Bacteriological analysis of fresh produce in Norway. *International Journal of Food Microbiology* 77: 199-204.

Kirby, R.M., Bartram, J. & Carr, R., 2003. Water in food production and processing: Quantity and quality concerns. *Food Control* 14: 283-299.

Kondro, W., 2000. *Escherichia coli* outbreak deaths spark judicial inquiry in Canada. *The Lancet* 355: 2058.

Lang, M.M., Harris, L.J. & Beuchat, L.R., 2004. Survival and recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on lettuce and parsley affected by method of inoculation, time between inoculation and analysis, and treatment with chlorinated water. *Journal of Food Protection* 67: 1092-1103.

Lapidot, A, Romling U. & Yaron, S., 2006. Biofilm formation and the survival of *Salmonella* Typhymurium parsley. *International Journal of Food Microbiology* 109: 229-233.

Lin, C.M. & Wei, C.I., 1997. Transfer of *Salmonella montevideo* onto the interior surfaces of tomatoes by cutting. *Journal of Food Protection* 60: 858-863.

Marino, D.D., 2007. Water and food safety in the developing world: Global implications for health and nutrition of infants and young children. *Journal of American Diet Association* 107:1930-1934.

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Millard, P.S., Gensheimer, K.F., Addiss, D.G., Sosin, D.M., Beckett, G.A., Houck-Jankoski, A. & Hudson, A., 1994. An outbreak of cryptosporidiosis from fresh pressed apple cider. *Journal of American Medical Association* 272: 1592-1596.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. & Tauxe, R.V., 1999. Food-related illness and death in the United States. *Emerging Infectious Diseases* 5: 607-625.

National Research Council, 1996. Public health concerns about infectious disease agents. In use of reclaimed water and sludge in food crop production, National Research Council, National academic press. www.nap.edu/books/0309054796/html/index.html. Accessed: 23 April 2008.

Ng, J.P., Graham, H.F. & Gillian, M.H., 2005. Pesticides as a source of microbial contamination of salad vegetables. *International Journal of Food Microbiology* 101: 237-250.

Nguyen-The, C. & Lund, B.M., 1991. The lethal effect of carrot on *Listeria* species. *Journal of Applied Bacteriology* 70: 479-488.

Richards, G.M. & Beuchat, L.R., 2005. Infection of cantaloupe rind with Cladosporium cladosporioides and Penicillum expansum and associated migration of Salmonella Poona into edible tissues. International *Journal Food Microbiology* 103:1-10.

Ryu, J.H., Deng, Y. & Beuchat, L.R., 1999. Behavior of acid-adapted and unadapted *Escherichia coli* O157:H7 when exposed to reduced pH achieved with various organic acids. *Journal of Food Protection* 62: 451-455.

Spasford, K.E., Rasooly, A., Taitt, C.R. & Ligler, F.S., 2004. Detection of *Campylobacter* and *Shigella* species in food samples using an Array Biosensor. *Analytical Chemistry* (76): 433-440.

© University of Pretoria

Steele, M. & Odumeru, J., 2004. Irrigation water as source of foodborne pathogens on fruit and vegetables. *Journal of Food Protection* 67: 2839-2842.

Takeuchi, K. & Frank, J.F., 2000. Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. *Journal Food Protection* 63: 434-440.

Takeuchi, K., Matute, C.M., Hassan, A.N. & Frank, J.F., 2000. Comparison of the attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Pseudomonas fluorescens* to lettuce leaves. *Journal of Food Protection* 63: 1433-1437.

Thunberg, R.L., Tran, T.T., Bennett, R.W., Matthews, R.N. & Belay, N., 2002. Microbial evaluation of selected fresh produce obtained at retail markets. *Journal of Food Protection* 67: 667-682.

Watkins, J. & Sleath, K.P., 1981. Isolation and enumaration of *Listeria monocytogenes* from sewage, sewage sludge and river water. *Journal of Applied Bacteriology* 50: 1-9.

Wei, C.I., Huang, T.S., Kim J.M., Lin, W.F., Tamplin, M.L. & Bartz, J.A., 1995. Growth and survival of *Salmonalla montevideo* on tomatoes and disifection with chlorinated water. *Journal of Food Protection* 58: 829-836.

Wonderling, L.D., Wilkinson, B.J. & Bayles, D.O., 2004. The htrA (degP) gene of *Listeria monocytogenes* 10403S is essential for optimal growth under stress conditions. *Applied and Environmental Microbiology* 70:1935-1943.

World Health Organisation, 2003. Eighth report of WHO surveillance program for control of foodborne infections and intoxications in Europe. Collab. Cent. Res. Train. Food hygiene zoonoses.

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www.who.int/foodsafety/publications/foodborne_disease/en. Accessed: 23 April 2008.

Young, K.D. & Thackston E.L., 1999. Housing density and bacterial loading in urban streams. *Journal of Environmental Engineering* 125:1177-1180.

Yu, K., Newman, M.C., Archbold, D.D. & Hamilton, T.R., 2001. Survival of *Escherichia coli* 0157:H7 on strawberry fruit and reduction by chemical agents. *Journal of Food Protection* 64: 1334-1340.

Zhuang, R.Y., Beuchat, L.R. & Angulo, F.J., 1995. Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied and Environmental Microbiology* 61: 2127-2131.

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Chapter 5: General discussions, Conclusions and Recommendations

Food safety control is a critical aspect both for consumer protection and the food industry, which needs to ensure consumer confidence. Therefore, food safety organisations and authorities have a very important role to play, ultimately serving the interests of the whole population and the economy (Kirby *et al*., 2003; Marino, 2007). Fruits and vegetables are often consumed raw or with minimal preparation. Raw agricultural products such as fresh produce might harbor a wide range of microorganisms including foodborne pathogens (Beuchat, 2002; Knabel *et al*., 2003). Contamination of fresh produce by foodborne pathogens may occur at any stage of production (Amoah *et al*., 2006; Izumi *et al*., 2008). Increased consumption of fresh produce over the past decade was accompanied by the increase in foodborne illnesses (Buck *et al*., 2003; Ilic *et al*., 2008).

Table grapes, like any other fresh produce rarely undergo minimal processing and have not been associated with the transmission of foodborne pathogens to human to date. This however does not mean that there is no risk of foodborne pathogen transmitted by table grapes. To assure safety of table grapes in the supply chain, it is important to identify possible sources and prevent microbial contamination during production and distribution. It was therefore, essential to study potential risks associated with current table grape production practices to ensure consumer protection against adverse food safety risks.

The use of contaminated agricultural water (for irrigation purposes or pesticide mixing) in the production of fresh produce has been linked to foodborne disease outbreaks (Hedberg *et al*., 1999; Cummings *et al*., 2001). In this study, on farm water sources and pesticides spray solutions were analysed to detect and quantify potentially pathogenic microbial populations of waterborne pathogens. Agricultural water sources were found to contain high numbers of faecal counts. However, the dectction of faecal indicators were dependent on the kind of pesticides used i.e. no faecal indicators were found in Dithane

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while the highest count were recorded in Teldor 500 EC alone. In a different pesticide trial of this study, Dithane showed the same inhibiting characteristics towards foodborne pathogens and that was in agreement with Guan *et al*. (2001) and Ng *et al*. (2005) studies. These studies showed that few pesticides inhibited the growth and survival of *E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium and *S. aureus.* The ability of foodborne pathogens to survive and multiply in pesticide mixtures was also shown by Guan et al. (2005).

Studies Ells and Hansen (2006) showed the ability of *L. Monocytogenes* to rapidly attach and colonise lettuce and cabbage surfaces. Under artificial post harvest simulated supply chain conditions, this study confirmed the potential of *E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium and *S. aureus* to attach, colonise and survive on table grape surface. The average time required for these foodborne pathogens to attach on grape skin ranged from immediately after contact to 60 s. Studies by Collignon and Korsten (2010) showed the ability *E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium and *S. aureus* to produce attachment structures on peach surface within 30 s to 60 s. Depending on the type of foodborne pathogen tested, colonisation of grape surfaces occurred after 15 min on average. Microscopic observations confirmed the formation of large micro-colonies or colonisation patterns near lenticels, on lesions and cracks. *Escherichia coli* O157:H7 and *L. monocytogenes* were able to survive under refrigeration conditions at - 2 ^{o}C which is in agreement with Glesson and O'Beine (2005).

Since the foodborne pathogens tested were shown to have the potential to attach to and survive on the grape surface, this may indicate some potential risk for table grapes coming into direct contact with contaminated water. Since the only direct water contact for table grapes may occur through mixing water for pesticides, some potential risk may be involved. However, given the inhibitory and bactericidal activity of some pesticides on microbial growth and survival, it may provide a practical solution to producers to reduce the potential risk. Therefore, this study suggests that a food safety risk may exist in situations where agricultural water is contaminated with pathogens such as

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E. coli O157:H7, *L. monocytogenes* and *S.* Typhimurium. However, water samples tested in this study did not contain any of these pathogens even though the water was considered highly polluted due to the Coliform and *E. coli* results. Using indicator systems may only provide presumptive information regarding the possible level of risk for the consumer at the end of the supply chain. The presence and persistence of *S. aureus* are more of importance in a handling perspective particularly during harvesting and packing. Up to date, there have been no effective interventions strategies developed which can completely eliminate food safety risks associated with the consumption of uncooked or unprocessed produce. Due to the potential of food safety risks and the recent *E. coli* outbreak in the European Union, it could be suggested that water microbial quality be more regularly tested and possibly treated prior to mixing of pesticides or selecting pesticides that can prevent pathogen growth and survival. Maintenance of standard hygiene practices could possibly reduce or prevent the spread of human pathogens to fresh produce.

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References

Amoah, P., Drechsel, P., Abaidoo, R.C. & Ntow, W.J., 2006. Pesticides and pathogen contamination of vegetables in Ghana's Urban Markets. *Archives of Environmental Contamination and Toxicology* 50: 1-6.

Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Journal of Microbes and Infection* 4: 413-423.

Buck, J.W., Walcott, R.R. & Beuchat, L.R., 2003. Recent trends in microbiological safety of fruits and vegetables. Plant health progress. www.cspinet.org/foodsafety/outbreak-alert.pdf. Accessed: 3 March 2008.

Collignon, S. & Korsten, L., 2010. 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. *Journal of Food Protection* 73: 1247-1256.

Cummings, K., Barrett, E., Mohle-Boetani, J.C., Brooks, J.T., Farrar, J., Hunt, T., Foire, A., Komatsu, K., Werner, S.B. & Slutsker, L., 2001. A multistate outbreak of *Salmonella* enterica serotype bail-don associated with domestic raw tomatoes. *Emerging Infectious Diseases* 7: 1046-1048.

Ells, T.C. & Hansen, L.T., 2006. Strain and growth temperature influence *Listeria* spp. attachment to intact and cut cabbage. *International Journal of Food Microbiology* 111: 34-42.

Gleesson, E. & O'Beirne, D., 2005. Effects of process severity on survival and growth of *Escherichia coli* and *Listeria innocua* on minimally processed vegetables. *Food Control* 16: 677-685.

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Guan, T.Y., Bank, G., Ismond, A. & Van Acker, R., 2001. Fate of foodborne bacterial pathogens in pesticide products. *Journal of the Science of Food and Agriculture* 81: 503-512.

Guan, T.Y., Blank, G. & Holley, R.A., 2005. Survival of pathogenic bacteria in pesticides solutions and on treated tomato plants. *Journal of Food Protection* 68: 296-304.

Hedberg, C.W., Angulo, F.J., White, K.E., Langkop, C.W., Schell, W.L., Stobierski, M.G., Schuchat, A., Besser, J.M., Dietrich, S., Helsel, L., Griffin, P.M., Mcfarland, J.W. & Osterholm, M.T., 1999. Outbreak of Salmonellosis associated with eating uncooked tomatoes: Implications for public health. The investigation team. *Epidemiology and Infection* 122: 385-393.

Ilic, S., Odomeru, J. & Lejeune, J.T., 2008. Coliforms and prevalence of *Escherichia coli* and foodborne pathogens on minimally processed spinach in two packing plants. *Journal of Food Protection* 71: 2398–2403.

Izumi, H., Tsukada, Y., Poubol, J. & Hisa, K., 2008. On-farm sources of microbial contamination of persimmon fruit in Japan. *Journal of Food Protection* 71: 52-59.

Kirby, R.M., Bartram, J. & Carr, R., 2003. Water in food production and processing: quantity and quality concerns. *Food Control* 14: 283-299.

Marino, D.D., 2007. Water and food safety in the developing world: Global implications for health and nutrition of infants and young children. *Journal of American Diet Association* 107:1930-1934.

Ng, J.P., Graham, H.F. & Gillian, M.H., 2005. Pesticides as a source of microbial contamination of salad vegetables. *International Journal of Food Microbiology* 101: 237-250.

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Knabel, S.J., Fatemi, P., Patton, J., Laborde, L.F., Annous, B. & Sapers., G.M., 2003. On farm contamination of horticultural products in the USA and strategies for decontamination. *Food Australia* 55: 580-582.

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6. Appendix

Table 2.1 Top 15 of table grape producers in the world and quantity exported

Countries	+Production /mt	+Area planted/ha	*Production/ kg/ha	*Export/t
World	6755199			
Italy	8242500	788100	98887	507448
China	8039091	423232	159645	63665
USA	6411660	378770	175302	424437
France	6101620	813496	69627	-
Spain	5573400	1109049	24280	136969
Turkey	4264720	482789	81162	202023
Chile	2500000	182000	131868	820716
Argentina	2184610	220000	131818	69718
India	1878000	68000	255147	118133
Iran	1876850	277747	62629	-
Australia	1797010	166197	117739	40785
South Africa	1703540	130000	137818	261519
Egypt	1550000	153956	99471	49740
Germany	1456000	99700	143307	39723
Brazil	1365490	79946	177798	82242

(+FAO, 2009; *FAO, 2008) Mt- metric tones; t - tones, _ - Data not found

Table 2.2 Top 15 importers of table grapes in the world

Countries	+Production/mt	+Area planted/ ha	*Import/t
USA	6411660	788100	527039
Russian	277880	41900	406760
Netherlands	125	50	356264
Germany	1456000	99700	305686
UK	1000	50	273074
Canada	80962	9575	192731
France	6101620	813496	163068
Poland	-	-	138008
China, SAR	-	-	89021
Mexico	317478	25956	81183
China	8039091	423232	78241
Ukraine	415300	70900	74569
Belgium	500	50	66319
Czech Republic	98323	16302	53596
Austria	1797010	399163	51588

(+FAO, 2009; *FAO, 2008) Mt- metric tones; t - tones, _ - Data not found

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Table 2.3 Examples of foodborne pathogens, associated diseases and duration of illness

Pathogen	Examples	Diseases	Duration of illness
Bacteria	Salmonella spp	Salmonellosis	Days – weeks
	Shigella spp	Gastroenteritis	4 – 7 days
	Escherichia coli	Urinary infection; diarrhoea	5 – 10 days
	Yersinia enterocolitica	Yersniosis (gastroenteritis)	Days – 3 weeks
	Campylobater jejuni	Campylobacteriosis	2 – 10 days
	Leptospira spp	Leptospirosis	Variable
	Mycobacterium spp	Tuberculosis; leprosy	Variable
	Clostridium botulinum	Diarrhoea	Days – month
	Clostridium perfringens	Diarrhea	24 – 48 hours
	Bacillus cereus	Bacillary dysentery	12 – 48 hours
	Vibrio cholera spp	Cholera	3 – 7 days
	Staphylococcus spp	Osteomylitis	24 – 48 hours
	Streptococus spp	Rheumatic fever	Variable
	Klebsiella spp	Pneumonia	Variable
	Brucella spp.	Rheumatic fever	Variable
	Enterobacter spp	Urinary tract infection	Variable
	Serratia spp	Meningitis; endocarditis	Variable
	Listeria minocytogenes	Listeriosis	Days – weeks
Viruses	Enteroviruses	Poliomyelitis	2 – 9 days
	Adenovirus	Systemic infections	2 – 9 days
	Reovirus	Acute respiratory infections	Variable
	Calicivirus	Respiratory infections	1 – 3 days
	Hepatitis A virus	Infectious hepatitis	2 weeks – 1 month
	Rotavirus & Norwalk	Acute gastroenteritis	4 – 8 days
	Astrovirus	Gastroenteritis	2 – 9 days
	Norovirus	Acute respiratory infections	1 – 3 days
Parasites	*Entaoeba histolytica*	Amoebiaisis	Variable
	Giardia lamblia	Giardiasis (gastroenteritis)	2 days – 2 weeks
	Cryptosporidium spp.	Cryptosporidiosis	4 – 21 days
	Arcaris spp.	Ascariasis	Variable
	Cyclospora spp.	Gastroenteritis	1 week – 1 month
	Taenia sp	Taeniasis	Variable
	Nector americanus	Ancyostomiasis	Variable
	Trichuris trichuria	Trichuriasis	Variable

CDC, 2000; CDC, 2002; CDC, 2009

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Table 2.4 List of registered pesticides and their maximum residue limits for grapes in South Africa

Pesticides (Active Ingredients)	South Africa	Holland	Canada	USA	United Kingdom	Germany	France	Export Default	Codex Aliment.	Europ. Union	Withholding Period/ Days
Acephate	1.5	0	0	0	0.02	0	1.5	0.02	0	0.02	30
Aldicab	0.2	0	0	0	0.05	0	0.2	0.02	0.2	0.02	120
Alphacyphermethrin	0.05	0	0	0	0	0	0	0.5	0	0.05	123
Azoxytrobin	1	2	3	1	2	2	1	2	0	1	14
Benalaxyl	2	0	0	0	0.2	0	0.2	0.2	0.2	0.2	56
Benomyl	1	0	0	0	0.3	0	0.5	0.3	0	0.3	42
Beta-cyfluthrin	0.1	0	0	0	0	0	0	0.3	0	0.1	35
Beta-cypermethrin	0.05	0	0	0	0	0	0	0.5	0	0.05	14
Boscalid	5	0	0	3.5	0	2	5	5	0	3.5	_
Bromopropylate	1	0	2	0	2	0	0	2	2	1	21
Captab/Cabtan	15	0.02	5	25	0.02	0.02	0.02	0.02	0	0.02	35
Carbaryl	2.5	0	5	10	0.05	0	0	0.05	5	0.05	42
Carbosulfan	0.05	0	0	0	0.05	0	0	0.05	0	0.05	_
Chlorphenapyr	0.5	0	0	0	0.05	0	0	0.05	0	0.05	_
Chloropyrifos	0.5	0.5	0.01	0.5	0.5	0	0.5	0.5	0.5	0.01	30
Copper Oxychloride	20	0	50	0	0	0	50	0.01	0	0.01	42
Cyanamide	0.05	0	0	0	0	0	0	0.05	0	0.05	_
Cycloxidim	0.05	0	0	0	0	0	0	0.05	0.05	0.05	_
Cyfluthrin	0.1	0.3	0	1	0.3	0.3	0.3	0.3	0	0.1	35
Cyhalothrin	0.2	0	0	0	0	0	0	0.01	0	0.01	_
Cymoxanil	0.1	0.05	0	0.1	0.1	0.2	0.1	0.2	0	0.05	42
Cypermethrin	0.05	0	0	0	0.5	0	0	0.5	0.5	0.05	28
Cyproconazole	0.1	0	0	0	0	0	0	0.2	0	0.1	_
Cyprodinil	0.5	3	2	2	3	2	1	5	3	0.5	28
Deltamethrin	0.1	0	0	0	0.2	0	0	0.2	0.2	0.1	7
Dichlofluanid	1	0	0	0	15	0	0	15	15	1	28 - 40
Dichlorvos	0.1	0	0	0	0.01	0	0	0.01	0	0.01	7
Difenoconazole	0.2	0	0	0	0	0	0	0.5	0	0.2	28
Dimethoate	2	0.02	_	1	0.02	0.02	0.02	0.02	0	0.02	14
Dimethomorph	5	0	0	0	0	0	3	3	0	5	56

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Table 2.4 Cont.											
Pesticides (Active Ingredients)	**South Africa**	**Holland**	**Canada**	**USA**	**United Kingdom**	**Germany**	**France**	**Export Default**	**Codex Aliment.**	**Europ. Union**	**Withholding Period/ Days**
Dinocab	1	0	0	0	0	0	0	0.05	0.5	0.05	14
Endosulfan	0.5	0.5	1	2	0.5	0.5	0.5	0.5	1	0.5	14
Esfenvalerate	0.05	0	0	0	0.02	0	0	0.02	0	0.02	_
Ethephon	5	1	1	2	1	0	1	1	1	1	_
Famoxadone	1	0	0	0	2	0	0	2	2	1	28
Fenamidone	1	0	_	1	_	0	0	_	_	0.01	49
Fenamiphos	0.05	0	0	0.1	0.02	0	0	0.02	0	0.02	
Fenarimol	0.2	0.3	0	0.1	0.3	0.3	0.3	0.3	0.3	0.2	21
Fenhexamid	5	5	4	4	5	5	0	5	15	3	42
Fenthion	0.5	0	0	0	0.01	0	5	0.01	0	0.01	10
Fenvalerate	0.05	0	0	0	0.02	0	0	0.1	1	0.02	14
Fluazifoppbutyl	0.05	0	0	0	0	0	0.02	0.2	0	0.05	_
Fludioxonil	0.5	2	1	1	2	2	0.5	2	2	0.5	28
Flurochloridone	0.02	0	0	0	0	0	0	0.1	0	0.02	_
Flusilazole	0.05	0	0	0	0	0	0.2	0.05	0	0.05	21
Folpet	15	0	0	0	0.02	0	0	0.02	10	0.02	42
Formetanate	0.05	0	0	0	0	0	0	0.05	0	0.05	_
Formothion	2	0	0	0	0.02	0	0	0.02	0	0.02	42
Fosetyl-Al	25	0	0	0	0	0	0	100	0	20	28
Gibberrellic Acid	0.2	0	0	0	0	0	5	5	0	0.2	_
Gamma-cyhalothrin	0.05	0	0	0	0	0	0	0.05	0.05	0.05	14
Hexaconazole	0.1	0	0	0	0.1	0	0	0.1	0	0.1	7
Imidacloprid	0.05	0	1.5	1	0	0	0	1	1	0.05	_
Iprodione	5	10	10	60	10	10	10	10	10	5	14
Iprovalicab	0.2	0	0	0	2	0	0	2	0	0.5	42
Kresoxim-methyl	0.5	1	1	1	1	1	1	1	1	0.5	14
Lambda-cyhalothrin	0.2	0	0	0	0.2	0	0	0.2	0	0.2	14
Mancozeb	3	0	0	0	2	0	2	2	0	2	28
Maneb	3	0	0	0	2	0	2	2	0	2	42
Mercaptothion/ Malathion	2	0.2	8	8	0.5	0.5	0.5	0.5	5	0.02	10

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Table 2.4 Cont.

Pesticides (Active Ingredients)	South Africa	Holland	Canada	USA	United Kingdom	Germany	France	Export Default	Codex Aliment.	Europ. Union	Withholding Period/ Days
Metalaxyl-M (Mefenonoxam)	1.5	2	1	2	2	2	2	2	1	1	**56**
Methidathion	0.2	0	0.2	0	0.02	0	0	0.02	1	0.02	21
Methiocarb	0.2	0	0	0	0	0	0	0.3	0	0.2	_
Metiram	3	0	0	0	2	0	0	2	0	2	14
Mevinphos	0.2	0	0	0.5	0.1	0	0	0.01	0	0.01	_
Myclobutanil	0.2	1	1	1	1	1	1	1	1	0.2	21
Nuarimol	0.05	0	0	0	0	0	0	0.01	0	0.01	21
Ofurace	0.2	0	0	0	0	0	0	0.01	0	0.01	_
Omethoate	1.5	0	0	0	0	0	0	0.02	0	0.02	_
Oryzalin	0.05	0	0	0.05	0	0	0.01	0.01	0	0.01	_
Oxadixyl	2	0	0	0	0	0	0.01	0.01	0	0.01	
Penconazole	0.2	0	0	0	0	0	0.2	0.2	0.2	0.2	14
Permethrin	0.5	0	2	0	0.05	0	0.05	0.05	2	0.05	14
Phosphorous acid	25	0	0	0	0	0	0	0.01	0	0.01	_
Piperonyl butoxide	5	0	8	8	0	0	0	0.01	0	0.01	_
Procymidone	5	0	5	0	5	0	0.02	5	5	5	28
Propiconazole	0.2	0	0	0	0.05	0	0.05	0.05	0.5	0.05	14
Propineb	3	0	0	0	2	0	0	2	5	2	42
Propoxur	0.05	0	0	0	0.05	0	0	0.05	0	0.05	_
Prothiofos	1	0	0	0	0	0	0.01	0.01	0	0.01	_
Propyzamide	0.1	0	0	0.1	0.02	0	0	0.02	0	0.02	_
Pyraclostrobin	0.5	1	0	2	1	1	1	1	2	0.5	42
Pyrethrins	1	1	1	1	0	1	1	1	0	1	_
Pyrifenox	0.1	0	0	0	0	0	0	0.01	0	0.01	35
Pyrimethanil	5	5	5	5	0	5	2	5	0	5	35
Quinoxyfen	1	1	0	0.6	1	1	1	1	0	0.5	21
Quintozene	1	0	0	0	0.02	0	0	0.02	0	0.02	-
Samazine	0.2	0	0	0.2	0	0	0.1	0.1	0	0.1	#VALUE!
Spinosad	0.01	0.2	0	0.5	0.5	0	0.5	0.5	0.5	0.01	14
Spiroxamine	1	0	0	0	1	0	1	1	0	1	42

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Table 2.4 Cont.											
Pesticides (Active Ingredients)	**South Africa**	**Holland**	**Canada**	**USA**	**United Kingdom**	**Germany**	**France**	**Export Default**	**Codex Aliment.**	**Europ. Union**	**Withholding Period/ Days**
Sulphur	50	0	0	0	0	0	50	50	0	50	42
Tebuconazole	2	2	5	5	0	2	0.5	2	2	0.5	42
Tetraconazole	0.5	0	0	0	0	0	0	0.1	0	0.1	14
Thiram	5	0	0	0	0	0	3	0.1	0	0.1	90
Triadimefon	2	2	0	1	2	2	2	2	0.5	0.5	14
Triadimenol	1	0	0	0	2	0	2	2	2	1	14
Trifloxystrobin	0.5	5	0	2	5	5	5	5	3	0.5	42
Vamidothion	3	0	0	0	0	0	0	0.05	0	0.05	_
Vinclozolin	3	0	_	_	_	0	0	5	5	3	42
Zeta-cypermethrin	0.05	0.5	0	2	0.5	0.5	0.5	0.01	0	0.01	14
Zineb	0	0	0	0	2	0	0.01	0	0	0	14
Zoxamide	2	5	3	3	5	5	5	5	0	0.5	42
Total No. of registered pesticides with MRL value	**102**	**102**	**99**	**101**	**100**	**102**	**102**	**101**	**101**	**102**	

(CODEX Alimentarius Commission , 2007; DAFF, 2009; Perishable Products Export Control Board, 2009) Note: _ Data not found; 0 - No residue required:

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