

SURVEY OF CURRENT WATER USE PRACTICES ON
FRESH FRUIT AND VEGETABLE FARMS AND
EVALUATION OF MICROBIOLOGICAL QUALITY OF SURFACE WATERS
INTENDED FOR FRESH PRODUCE PRODUCTION

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Elizabeth Ann Bihn, Ph. D.

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Fruits and vegetables are a delicious and nutritious food source enjoyed worldwide. Most produce is grown in fields, under open skies, where human pathogens could be present and then transferred to fresh produce during production, harvesting, and packing resulting in contamination. Consumption of contaminated fresh produce can result in produce-associated foodborne illnesses as has been documented multiple times over the last three decades in commodities such as spinach, tomatoes, lettuce, melons, and peppers to name a few. Fresh fruits and vegetables that are eaten raw, receive no treatment that would remove or kill bacteria, viruses or other microorganisms that may be present. Thus the focus for reducing produce-associated foodborne illnesses is on preventing contamination before it occurs. Understanding risks that exist on farms and in packinghouses and developing Good Agricultural Practices (GAPs) that reduce these risks are critical to preventing contamination. The studies in this dissertation focus on fruit and vegetable production as it relates to food safety, with an emphasis on water management practices

especially from surface water sources. Specifically, a grower survey was conducted to assess on-farm practices related to the use of surface water sources during the production of fruits and vegetables because surface water represents a potential microbial hazard, particularly if it is applied directly to the edible portion of the plant during irrigation, frost protection or the application of protective topical sprays. Samples from surface water sources on farms throughout New York and Tennessee were analyzed for water quality indicators such as quantified generic *E.coli*, specific conductance, turbidity, and pH with a subgroup of samples analyzed for *Salmonella spp.* as another means of assessing risk. This resulted in a better understanding of produce safety issues, particularly those related to the use of surface water during production to guide the practical implementation of food safety practices on farms and in packinghouses based on current, relevant scientific data. Reducing contamination risks through science-based risk assessment and the implementation of GAPs to reduce identified risks are effective and practical approaches that can be utilized by all growers to help ensure safe fresh fruits and vegetables.

BIOGRAPHICAL SKETCH

Elizabeth A. Bihn was born in Perrysburg, OH to Mary Ann and Jerry Bihn. She is the youngest of four children. Betsy received her Bachelor of Science degree in 1994 from Ohio State University in zoology and a minor in plant biology. She received her Master of Science degree in 1997 from the University of Florida in horticulture. Betsy is married to Courtney A. Weber and has two sons, Rye and Luke.

To Rye and Luke, whom I love and treasure beyond comprehension
and
To Courtney, my brave husband, who did not give up or run away!

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It seems I always save the acknowledgments for last, hoping that I will be able to capture what needs said in some precise and concise manner, making certain to not forget anyone. Unfortunately, I know this is not possible, so I will do the best I can and know anyone who is not interested in reading will simply turn the page. I am keenly aware that my success in completing this degree and dissertation is not mine alone. My husband, Courtney, not only provided love, support, and the final frustrated demands that I finish, but also exceeded all parenting and house-keeping expectations by picking up all the slack left by a preoccupied and stressed wife. There would have been a whole lot less slack without the other two small men in our lives, Rye and Luke. Though they add to the work load, they more than make up for it with their loving dispositions and habits for unbridled laughter. They tolerated the process and developed a whole vocabulary foreign to most kids (pathogenic microorganisms, dissertation, *Esherichia coli*...you get the point). My parents not only donated genetic material but have provided endless love and support my entire life. Most people do not get to pick their parents, so I consider myself truly blessed to be born into my family. My siblings, my in-law siblings, and my in-law parents not to mention extended families on all sides have offered steady encouragement and help with a special shout out to my sister Cheryl for all the free medical advice a crazed graduate student could need. I have never known anything but a big family so I cannot really imagine anything else but I have a hard time believing there are better families to be a part of than the one I was born to and the one into which I married.

This, of course, says nothing of the friends that are the chosen family. Forgive the redundancy, but blessed is the only word that even comes close to describing how I feel about my friends. How do you acknowledge calls and advice that run from the

fears my children's illnesses can inspire or caring for my dying dog through debating the values of prescription anxiety meds and any other sensitive, overwhelming subject that happens into my life. Pile on graduate school and it seems many of my friends should have been put on paid retainer for their help, friendship and guidance. It is impossible to name all my friends who contributed to the cause that is my life, but Nancy and Audrey did some pretty heavy lifting that more than once saved me from emotional ruin. This is not to say that all friendships involved heavy lifting, unless you consider 12 ounces heavy. My friends not only bring support, but lots of joy and laughter. I really cannot imagine where I would be without them as they provide a texture to my life that is not replaceable or even describable (you all know who you are!).

Stepping towards the more academic side of life, I cannot help but trip over my friend and committee chair, Randy. It is hard to tell if he actually knew what he was getting into when he agreed to accept me as a graduate student in his lab. How can anyone know the process will take seven...yes, seven...years? A good advisor but more important to me, a great friend who kept his advisor foot at the ready anytime I needed a swift kick in the pants. Committee member Steve gets the award for bravery as a co-author, and consistent concern with the ever present knock on my door to ask "how are you doing?". Committee member Bob gets the endurance award for working with me for 12 years of which 7 I have been in graduate school...ouch! And in my final finger pointing at Cornell faculty, Terry Acree put the silly notion in my head that I should enroll in school to complete my Ph.D. Dude, what were you thinking?

This seven year process eventually had to end with a completed dissertation and as it turns out, I could not manage to do that by myself either. There were the New York State Department of Agriculture folks and an illegally driving summer student who helped collect and analyze samples as well as colleagues in other states

who participated in the database by adding more data points. Don spent way too many of his weekend hours with me discussing statistics and Jud donated his PCR skills and patience when my defense was on the calendar and my brain too stretched to know up from down. Finally, Mark who not only made the database a reality (it's ALIVE) but also has been a presence in my life ever since I arrived at Cornell making sure anything I created in the printed word was formatted correctly and looked divine. If you think any of this sounds easy, you have clearly never worked with me!

In finishing, it's hard for me not mention Robin Roffey and Dick Sayre. It seems life would have turned out much differently had Robin not recruited me to wash dishes in Dick's lab during my freshman year at Ohio State. Maybe I would have found my way to science on my own, but their influence on me is undeniable for better or worse, and I tend to think for the better.

Although I know there is much more, that will have to be it for now. With all my spare time, I might just have to write a book if I want to share the whole story. The take home is that I did not do this by myself. I have been abundantly blessed.

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Chapter One
Produce Food Safety and Good Agricultural Practices
During Fresh Fruit and Vegetable Production

Introduction

Everyone is affected by the safety of food. Its impact on the daily lives of individual consumers is important but food safety also impacts those involved in the production of food. Some foods are processed according to validated and verified protocols essentially guaranteeing the safety of the food product before it is sold or it is sold raw but cooked in the home before being consumed. Other foods, such as fresh fruits and vegetables, are consumed while they are still raw and in their native form. Fresh produce that is consumed raw receives no treatment to kill or destroy pathogenic microorganisms that may be present on the surface or the interior.

Over the past several decades, fresh produce (fruits and vegetables) consumption has been increasingly linked to foodborne illness outbreaks due to unintentional contamination with enteric human pathogens (USFDA, 2001; Sivapalasingam et al, 2004; Vierk, 2008). Unintentional contamination by *E. coli* O157:H7, *Salmonella*, *Cyclospora*, and Hepatitis A accounted for 96% of the outbreaks and 95% of the illnesses in reported produce related outbreaks from 1996-2007 (Vierk, 2008). Contamination of fresh and fresh-cut fruits and vegetables with pathogens can occur anywhere in the supply chain and once it occurs is difficult, if not impossible, to remove (Beuchat and Ryu, 1997; Gagliardi et al, 2003; Doyle and Erickson, 2008). There are some developing technologies such as irradiation that may offer protection in

Significant portions of this chapter were previously published in Bihn, E.A. and S. Reiners. 2010. Good Agricultural Practices and Good Manufacturing Practices for Vegetable Production. In *Handbook of Vegetables and Vegetable Processing* (pp461-481). Wiley-Blackwell, John Blackwell and Sons Publishing Co., Ames, Iowa.

the future, but at this stage, preventing contamination and controlling multiplication if contamination by microbial pathogens occurs, are the most effective approaches.

Fresh fruits and vegetables grow in areas where human pathogens could be present and then transferred to fresh produce resulting in contamination. Among these areas, the most likely potential mechanisms of contamination by human pathogens such as *E. coli* O157:H7 and *Salmonella* include soil amendments (i.e., manure, compost and compost teas), water (i.e. irrigation or flooding/runoff from adjacent and protective sprays), direct contact with wildlife, airborne deposition from off-farm activities such as cattle/dairy and manure/composting operations, and postharvest handling (Beuchat and Ryu, 1997; Beuchat, 2002; Aruscavage et al, 2006; Brandl, 2006). Produce outbreak investigations by local, state, and federal regulatory authorities have linked pathogen contamination to the field or postharvest handling environment, but have rarely provided definitive evidence identifying exactly what factor or factors lead to the unintentional contamination (CDC, 1997; Hilborn et al, 1999; Herwaldt, 2000). More commonly, investigators compile lists of suspected risk factors that most likely contributed to the contamination event. Even when researchers deliberately expend time and effort looking for specific human pathogens in the field, they often cannot locate them (Riordan et al, 2001). These data indicate that produce contamination may occur by a multitude of means, and no one means can account for contamination even among specific produce pathogen pairings such as *E. coli* O157:H7 contamination of leafy greens or *Salmonella* spp. contamination of tomatoes.

Growing and field conditions are highly variable and can be dramatically different between growing regions, resulting in inconsistent rates of contamination (Mukherjee et al, 2007). Furthermore, practices used to grow, harvest and pack even one crop may have a multitude of variations even within any given growing region resulting in different microbial

risks (Mukherjee et al, 2007). These situations are compounded by the hundreds of commodities being grown with different physiological traits, that make them more or less likely to be contaminated (Stine et al, 2005). Continued high profile foodborne illness outbreaks associated with produce consumption have increased the pressure on fresh produce growers to implement food safety practices and document their food safety program for external entities such as buyers or third party auditors. Recent peer reviewed produce food safety research from academic and government institutions around the world have found numerous new potential sources, vectors and means of unintentional contamination of produce (Doyle and Erickson, 2008; Izumi et al, 2008; Miller et al, 2008; Orozco et al, 2008). This new scientific data coupled with a lack of definitive information as to the causes of recent produce-associated foodborne illness outbreaks creates a problem for produce growers and postharvest handlers. The pressure to develop, implement, and document produce safety practices is high, but it is not always evident what practices will most effectively reduce the risks that exist. It is particularly frustrating when current scientific research cannot provide a clear and decisive road map to guide the implementation of food safety practices.

This dissertation will focus on fruit and vegetable production as it relates to food safety, with an emphasis on water management practices focused on surface water sources. The goal is to increase understanding of produce safety issues, particularly those related to the use of surface water during production and guide the practical implementation of food safety practices on farms and in packinghouses based on current, relevant scientific data. Protecting fruits and vegetables from contamination is as complex as the food system that is required to grow, harvest, store, transport, and market the commodities. In addition, there are risks introduced by consumers themselves. Since growers cannot control the safety of fresh produce throughout the entire food

system, they must focus on identifying and controlling risks that exist on the farm and in the packinghouse. Managing food safety risks at the farm level takes place within the greater farm management structure that must include crop production and protection, personnel management and training, marketing, and effective reaction to the ever variable and uncontrollable weather conditions. This literature review has significant focus placed on Record Keeping; Worker Health, Hygiene, and Training; Soil Amendments and Manure; Production Water; Wildlife; Postharvest Water; Cleaning and Sanitation; Pest Control; Traceability and Recall; and Crisis Management. These are key areas that have been identified as significantly important through Delphi studies conducted for Good Agricultural Practices (GAPs) related educational programs. In addition, there will be an attempt made to clarify the terminology used in produce safety with respect to the food safety continuum, from farm to fork. This effort is due to the fact that inconsistent terminology use has resulted in undue stress and confusion for many fresh produce growers as indicated by numerous extension phone calls and questions from growers.

It is important to mention that fresh fruits and vegetables have the potential to play a defining roll in the overall health of individuals. The trend of increased obesity in the United States increases diseases such as cardiovascular disease, type 2 diabetes, and stroke risk. A diet rich in fresh fruits and vegetables can protect against cancer, cardiovascular disease and diabetes, with research indicating the more fresh produce an individual consumes, the more protection they gain (Donaldson, 2004). Consumption of more fresh produce would not only be good for individuals, but aid in the reduction of costs associated with health care. The Achilles' heel of the fresh produce industry is produce-associated foodborne illnesses. Foodborne outbreaks decrease immediate consumption and sales of commodities that are associated with the outbreak, but also have long term impacts (Arnade et al, 2009). Research that allows for the development

of science-based practices that effectively reduce microbial hazards in fresh produce will be good for consumers and the produce industry.

Clarifying the Language of Produce Safety

If produce safety is viewed as a continuum from farm to table, Good Agricultural Practices (GAPs) would be the first step. The concept of GAPs was introduced in the 1998 publication by the Food and Drug Administration, Center for Food Safety and Applied Nutrition entitled “*Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*”. This guide focuses on risk reduction, not risk elimination because “current technologies cannot eliminate all potential food safety hazards associated with fresh produce that will be eaten raw” (USFDA, 1998). Since microbial contamination is difficult to remove once fruits and vegetables are contaminated, the focus of GAPs is prevention. Fresh produce growers and packers directly impact safety through their actions and through the implementation of produce safety practices such as GAPs. The foundation of any produce safety program is a company-wide commitment that extends from the farm owner to every farm employee. Everyone in the produce operation impacts safety, so everyone needs to understand their role in the implementation of food safety practices.

Good Agricultural Practices

Food safety begins on the farm. GAPs are any agricultural management practice or operational procedure that reduces microbial risks or prevents contamination of fruits and vegetables on the farm or in the packinghouse. GAPs are not one set of defined practices, but provide latitude for every fresh produce grower to implement their own practices to prevent or minimize risks, because each operation is unique and its practices may differ depending on many

variables including cultural practices, location, and commodities grown. GAPs focus on field production including such areas as soil amendments, irrigation water sources, field packing, transportation, and worker training. As production and handling of fresh produce moves into areas where the level of control is higher than a field environment, Good Manufacturing Practices (GMPs) can be applied. The flow from GAPs to GMPs is a smooth transition with many of the same concepts and areas addressed in both.

Good Manufacturing Practices

Good Manufacturing Practices (GMPs) in manufacturing, packing, or holding human food are codified in the Code of Federal Regulation, Chapter 21, Part 110. Currently fresh fruits and vegetables are exempt from this legal code as stated under Exclusions “(a) The following operations are not subject to this part: Establishments engaged solely in the harvesting, storage, or distribution of one or more “raw agricultural commodities” as defined in section 201(r) of the act, which are ordinarily cleaned, prepared, treated, or otherwise processed before being marketed to the consuming public. (b) FDA, however, will issue special regulations if it is necessary to cover these excluded operations” (21 CFR part 110). Even though fresh produce is currently exempt from the regulation, GMPs outline important practices that should be followed to reduce chemical, physical, and microbial hazards that may be present in packinghouses, greenhouses, or other buildings with doors, windows, and screens that offer a level of control that is absent in field environments. There has also been discussion of removing this exemption, so it is important to understand GMPs and how they are applied to food production facilities. Subpart headings present in the GMPs include:

Subpart A: General Provisions

110.3 Definitions

110.5 Current Good Manufacturing Practices

110.10 Personnel

110.19 Exclusions

Subpart B: Building and Facilities

110.20 Plant and grounds

110.35 Sanitary operations

110.37 Sanitary facilities and controls

Subpart C: Equipment

110.40 Equipment and utensils

Subpart E: Production and Process Controls

110.80 Processes and controls

110.93 Warehousing and distribution

Subpart G: Defect Action Levels

110.110 Natural or unavoidable defects in food for human use that present no health hazard

Subparts D and F: Reserved

Throughout this chapter, GMPs relevant to the production and packing of fresh vegetables will be discussed. There is some level of overlap between GAPs and GMPs, and since fresh produce operations are currently exempt from implementing GMPs, there was not a significant effort made to specifically categorize actions as one or the other. Effort was placed on explaining risks that may be present and providing examples of implementation during fresh fruit and vegetable production and packing to reduce identified risks.

HACCP

As produce moves into more complex systems such as fresh-cut operations or processing plants, the application of Hazard Analysis Critical Control Point (HACCP) is appropriate (Figure 1-1). This multi-faceted, seven principle process includes conducting a hazard analysis (principle 1) to identify the risks that exist in processing, packaging, and selling foods (NACMCF, 1998). Once the hazards are identified, critical control points (CCPs) are established (principle 2) to prevent or eliminate the food safety risks. These CCPs define procedures where control can be applied and food safety hazards can be prevented, eliminated, or reduced to acceptable levels. There are five additional steps required to develop a full HACCP program including establishing critical limits (principle 3), establishing monitoring procedures (principle 4), establishing corrective actions (principle 5), establishing verification procedures (principle 6), and establishing record-keeping and documentation procedures (principle 7). Rushing et al (1996) reported on a HACCP program established in a fresh-market tomato packinghouse where a level of control over the operation was established and three CCPs were identified. This same level of control is not attainable at the field level, which is why applying HACCP at the field level is difficult, if not impossible.

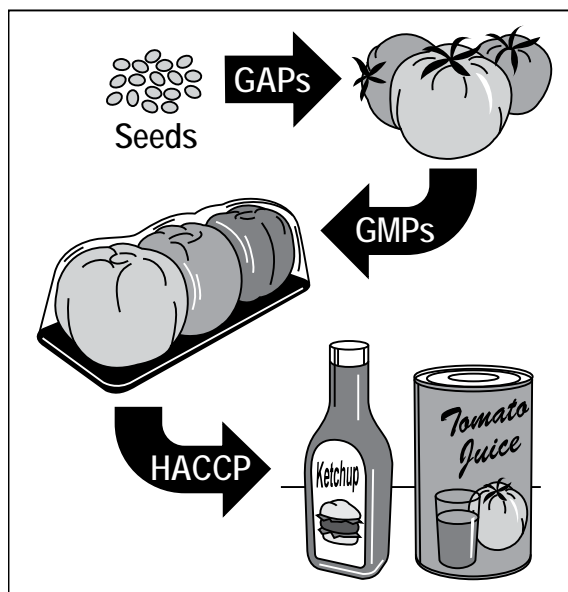


Figure 1-1. The progression of food safety programs from the field to the processing plant: Good Agricultural Practices (GAPs) in the field, Good Manufacturing Practices (GMPs) in the packinghouse, and Hazard Analysis Critical Control Point (HACCP) in the processing plant.

This last statement is hotly debated, as some feel the application of HACCP at the field level is completely appropriate even if no CCPs are identified or can be established. The importance of including HACCP in this discussion is to make the argument that what really matters is implementing practices that improve food safety and defining expectations that are clearly communicated in a manner that is understandable throughout the food system. Traditionally, food processors are more familiar with food safety practices, including HACCP, than primary producers. As foodborne illness outbreaks and the desire to develop system-wide traceability have increased, buyers have started to request their suppliers follow food safety practices to ensure the product they are acquiring is safe. This results in contracts and discussions where food safety language is included and buying requirements established. When contracts require farms to have a HACCP plan, it can create confusion because it is not clear what CCPs should be established. As an example, consider the risk that wild deer present.

Fields are open to wildlife 24 hours a day, 7 days a week, 365 days a year. Deer are known vectors of *E. coli* O157:H7 and they have access to production fields, so they clearly represent some risk (Sargeant et al, 1999). Some level of control can be achieved, but to date there is no deer proof fence or 24 hour armed guard system on farms. What would be the critical limit for number of deer in a field? What would be the corrective action if you exceeded the set limit? How would you know you exceeded the set limit? For the sake of this dissertation, this argument could also be applied to irrigation water quality. Though there are currently no national irrigation water quality standards, industry groups such as the Leafy Greens Marketing Agreement have adopted standards based on the Environmental Protection Agency Recreational Water Quality Standards, which assume some level of resulting illness due to full body contact with the water (USEPA, 1986; CSFSGPHLLG, 2010). Irrigating with this water does not result in full body immersion of consumers, but the water is not free of microbial contaminants and therefore does pose a risk. It is also not clear if illness is likely to occur and currently there is little science that is applicable to all commodities in all regions of the country. The point of this discussion is that an appropriate hazard analysis that might result in identifying critical control points is extremely difficult.

The goal of all food safety programs is to improve safety by reducing or eliminating risks through the implementation of effective practices. Eliminating all risks on the farm is not realistic at this point but reducing risks is certainly achievable (USFDA, 1998). To achieve risk reduction at the farm level, it is important to provide guidance that is realistic and that resonates with those who must implement it, namely the growers. Promoting a system such as HACCP that requires a distinct level of control in a production system that is open and not feasibly

monitored 24 hours a day, adds confusion and frustration that is not productive and does not lead to the implementation of food safety practices that reduce relevant risks.

Record Keeping

Beginning the discussion of GAPs with record keeping is a bit counter intuitive. If a grower does not have any practices implemented, certainly they will not be keeping records of something that does not exist. Outlining record keeping is a logical first step in the process because if practices are implemented without record keeping in mind, establishing a record keeping system that is efficient and effective will be very difficult, resulting in the failure to record anything. The record keeping mantra is “if it is not written down, it did not happen.” Record keeping is important for growers and packers because it allows them to follow their progress in implementing and updating produce safety practices and it is required if the farm or packinghouse needs to have a third party audit to verify produce safety practices. In the event of a foodborne illness outbreak, record keeping will allow growers to provide detailed information to inspectors and show due diligence in the implementation of produce safety practices.

Development of a detailed farm food safety plan is the first step to good record keeping. Farm food safety plans should include information about the farm including total acreage and commodities grown, but can also include farm history and farm philosophy. This plan should include a farm-wide risk assessment and the farm practices that are used to reduce the identified risks. Standard Operating Procedures (SOPs) should be included in the plan and outline critical actions to ensure they are done properly and result in effective risk reduction. All actions taken to implement the farm food safety plan should be documented including, but not limited to, worker training, water testing, manure applications, cleaning and sanitation practices, and pest control activities.

There are many resources available to help growers develop and implement their farm food safety plans including a template food safety plan from the University of Minnesota at <http://safety.cfans.umn.edu/>. Template record keeping sheets are available for free download from the National GAPs Program at <http://www.gaps.cornell.edu/rks.html>. The sheets can be readily modified to meet individual farm needs and to include company logos if so desired. In addition to developing a farm food safety plan and record keeping sheets, growers need to establish record keeping schedules that are relevant for the implemented practices and make the record keeping sheets accessible to those in charge of record keeping. Clipboards that have pens tied to them or plastic sheet covers taped on three sides can be effective tools for placing record keeping sheets in convenient locations that help ensure record keeping is done in an efficient manner. Time wasted looking for record keeping sheets and pens are a waste of valuable human resources and can result in inconsistent record keeping practices.

A system for maintaining and retaining records should be developed so that they remain up-to-date and accessible. The National GAPs Program suggests maintaining records for a minimum of two years, although keeping records longer may be required by law or desirable for programs such as organic certification. The record keeping system also should include how to properly dispose of records since this will keep relevant records more organized and reduce the need for additional storage space.

Worker Health, Hygiene, and Training

Workers' health and hygiene practices cannot be over emphasized when addressing produce safety. Most fruits and vegetables that are consumed raw are harvested by hand. In addition to being harvested, most packing facilities include at least one hand culling step and result in hand packing of the commodities into boxes that have been folded by hand. Anything

that is on the hands of those who are involved in harvesting, culling, packing, or making boxes can be transferred to the fresh produce. These include viruses, bacteria, and parasites. Workers can shed pathogenic microorganisms through their urine, feces, saliva, and nasal mucus (Todd et al, 2008). In a documented hepatitis A outbreak that resulted from the consumption of fresh blueberries traced back to a single commercial orchard, it was noted that the operation did not have a method of recording worker illnesses and did not provide proper hand washing facilities in the field for its workers (Calder et al, 2003). This outbreak resulted in 81 laboratory confirmed cases of hepatitis A with 18 patients hospitalized and one death. The impact of developing and implementing a worker hygiene policy and providing proper facilities so that workers can practice proper hygiene is critical to both produce safety and worker health.

Proper facilities that are stocked with toilet paper, soap, water and paper towels must be provided for workers, otherwise there is no way for workers to protect themselves or the fresh produce they harvest and pack. Since hand washing facilities need to be provided in the field, many growers have asked about the need to provide warm or hot water for hand washing. Research indicates that the temperature of the water does not have a significant effect of the effectiveness of hand washing (Michaels et al, 2001). A key to successful hand washing is active scrubbing, so proper training is far more important than providing warm water. All toilets should have toilet paper provided. Toilet and hand washing facilities must be monitored to ensure they are well stocked and cleaned on a regular basis. Providing clean, well-stocked facilities shows company commitment to the produce safety program and provides workers an opportunity to actively participate in the produce safety plan and reduce risks.

It is particularly important to mention that hygiene practices such as hand washing must be practiced by all employees, including farm owners, managers, crew leaders, and facility

guests. If company policy states that all employees are required to practice proper hand washing, but farm owner and managers do not adhere to these practices, the policies and training programs for workers become meaningless. When those who make the policy do not follow the policy, it demonstrates to other employees that the policy is not important. Although most people report washing their hands, in one study 60% of food service personnel were observed to not wash their hands after using the toilet (Emery, 1990). Knowing that foodborne illness causing organisms can be transmitted to fresh fruits and vegetables during handling should make worker training programs critically important in all fresh vegetable operations and a company-wide priority.

Every farm and packinghouse operation should have a written illness reporting protocol. Any worker who is ill should report the illness, it should be documented, and the worker should be sent home or placed in a job where there is no chance of contaminating the fresh produce, equipment, packaging materials, or other workers. Since many agricultural workers do not receive paid sick leave, they may be hesitant to report illnesses if it results in being sent home without pay. Training needs to clearly outline the company illness reporting policy and explain the risks to fresh produce, as well as to the workers, if they continue to work while they are ill. In addition, supervisors should be trained to recognize signs of illness such as frequent trips to the bathroom, so they can assist employees who may be ill, encourage them to report illnesses, and seek medical attention when warranted. It is important to keep ill workers away from fresh produce to reduce the risks that they represent to the products and other workers.

A comprehensive worker hygiene training program is critical to food safety so that all employees understand the importance of proper hygiene. Proper hygiene includes bathing daily, wearing clean clothes to work, practicing proper hand washing after using the toilet, before and after work, after taking breaks, before and after eating, and any time employees come into

contact with dirty surfaces or substances. When produce-associated outbreaks are caused by Hepatitis A or *Shigella*, the source of the pathogens is almost always produce handlers since humans and other primates are the main reservoirs for these pathogens (Fiore, 2004; Wheeler et al, 2005; USFDA, 2009). The foodborne pathogens are transferred through the fecal-oral route highlighting the risk that ill workers pose. In addition to spreading human pathogens when they are sick, workers can also serve as a vehicle to spread human pathogens from a contaminated surface to fresh produce through handling. For all of these reasons, employee hygiene practices are important to fruit and vegetable safety, so implementing an effective worker training program describes and encourages proper hygiene practices that reduce these risks.

Worker training programs must be very specific about expectations with desired practices and enforcement procedures described in detail. It is most effective to allow workers to practice the behaviors while a trainer is present so that they can receive positive reinforcement when practices are performed correctly and further training when done incorrectly. Some practices, such as disposing of used toilet paper in the toilet, are not things that are easily practiced in the company of others, but those practices can be monitored by checking toilet facilities throughout the day to ensure toilet paper is not being disposed of in the garbage can or on the floor.

The toilet paper disposal issue bears discussion because it continues to be an issue in many operations. Improper disposal of toilet paper is problematic on many different levels. First, it disrupts the use of toilet facilities by creating unpleasant smells and unsanitary conditions if it is deposited on the floor or in cardboard boxes. These unsanitary conditions can attract insects which can transport contamination to other areas including foods. In addition, used toilet paper thrown on the floor can contaminate the floor and be moved from the toilet area on shoes resulting in direct fecal contamination of food production areas including fields and

packinghouses. This particular issue is also grounds for an automatic failure of some third party food safety audits, including the USDA Good Agricultural Practice/Good Handling Practice (GAP/GHP) audit.

Workers who do not properly dispose of used toilet paper, likely do so because of past experiences with plumbing that was not sufficient to handle toilet paper waste. Most facilities in the United States have either indoor plumbing or portable toilet facilities that are sufficient to handle toilet paper waste, but workers need to be explicitly told that this is the appropriate practice. Since workers are adults, it is most effective to use the concepts of adult learning and explain in detail what is expected (throw used toilet paper in the toilet) and why the action is appropriate (toilets in the US are made to have used toilet paper deposited directly into them and it is a food safety problem if toilet paper is deposited on the floor or in the garbage can).

All company policies regarding illness reporting and worker hygiene practices should be outlined in the farm produce safety plan. All employee training should be documented by identifying the training content, name of trainer, date of training, and a list of all employees who attended the training. Every employee should be trained prior to starting work. This can be a challenge for operations that bring on additional harvest and packing crews in the middle of the season since it is the busiest time of the year. Having a well outlined policy and training program will allow for the proper implementation of worker training programs to reduce microbial risks that exist from direct hand contact of fruits and vegetables. Conducting training programs, providing proper hygiene facilities such as toilets, toilet paper, sinks, water, soap, and paper towels, and enforcing implementation of company policies is relatively inexpensive and significantly reduces food safety risks during the production and packing of fresh fruits and vegetables.

Soil Amendments and Manure

Soil amendments are used to add organic and inorganic nutrients to the soil as well as improve soil tilth and fertility. Synthetic fertilizers such as urea, diammonium phosphate, and potash do not pose a microbial risk because they contain no animal products. Other soil amendments like limestone, gypsum, and rock powders are also safe from a microbiological perspective. These soil amendments still require proper management to avoid negative impacts on the crop and the environment. All soil amendments must be stored, handled, and applied as specified on the label or based upon production recommendations to protect the crop, environment, and people handling the materials.

Soil amendments containing raw animal manure can come from a variety of sources including cows, pigs, horses, and chickens and their bedding. It can be liquid, solid, or combined into slurry. All manure can carry foodborne pathogens and needs to be managed to prevent the microbial contamination of fresh fruits and vegetables. Recommended timing for a raw manure application prior to harvest varies from 90 days to five years. The National Organic Program (NOP) requires that manure be incorporated into the soil not less than 90 days prior to the harvest of a product whose edible portion does not have direct contact with the soil surface or soil particles, or 120 days prior to the harvest of a product whose edible portion has direct contact with the soil surface or soil particles (NOP rule 7 CFR Part 205.203) (NOP, 2000).

The Commodity Specific Food Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens states the best practice as “DO NOT USE raw manure or soil amendment that contain un-composted, incompletely composted or non-thermally treated animal manure to fields which will be used for lettuce and leafy green production” (CSFSGPHLLG, 2010). Florida T-GAPs developed for tomatoes, allow only properly composted manures to be

used in tomato fields and greenhouses, and stipulates that records of dates of composting, methods utilized, and application dates must be documented (FDACS, 2007). Some leafy greens buyers have required growers to sign contracts that state that leafy greens fields have not had manure applied in the last five years, so both industry groups and buyers impact expectations regarding manure use.

Foodborne pathogen survival and multiplication in soil is affected by soil type, tillage practices, commodity grown, and nitrogen availability, moisture content (Gagliardi and Karns, 2000; Islam et al, 2005). A five-year pre-harvest period for raw manure application may be extreme, but studies have found that microbial pathogens such as shiga-toxin producing *E. coli* strains can persist in soils up to 18 weeks, which exceeds both the 90 and 120 day application recommendations (Fukushima et al, 1999). *E. coli* O157:H7 survived for up to 196 days in amended field soil, and was detected on the surface of carrots 168 days after application of spiked compost (Islam et al, 2005). Side-dressing crops with raw manure or with straw bedding from animal operations should be viewed as a raw manure application and managed accordingly. Understanding that foodborne pathogens can survive and multiply in soil and that animal manures can contain foodborne pathogens explains why composting or other manure treatment options such as thermal processing prior to application to fresh produce fields reduces microbial foodborne pathogen risks.

If manure will be composted on the farm, proper composting protocols should be followed. Cornell Waste Management Institute (CWMI) provides many resources to assist with the establishment and management of compost piles. Composting is an active process that requires management such as establishing an initial Carbon:Nitrogen ratio of between 25:1 to 40:1 and maintaining the temperature between 131°F and 170°F for 15 days in a windrow

composting system with a minimum of five turnings (NOP, 2000). A United States Department of Agriculture Task Force set forth composting recommendations that are more flexible than those listed above and require that compost reach a minimum of 131°F for three days with sufficient management to ensure all parts of the pile reach this minimum temperature (CWMI, 2004). The Compost Fact Sheet Series (#1-8) provides a good foundation of information and is supported by many other CWMI resources at <http://cwmi.css.cornell.edu/resources.htm#composting>. Some organic certifiers, produce buyers, and commodity groups may have other requirements, so it is important to verify practices before compost is applied to fields containing fresh produce crops.

There are other parameters to consider when establishing compost piles. Domestic and wild animals should be actively excluded from the composting area to prevent recontamination of the compost. Compost piles should be located downhill or at a sufficient distance from fruit and vegetable fields to assure that rain does not lead to run-off contamination of fresh produce fields. Distance is important because wind can also present a contamination risk. It is difficult to establish precisely set distances or exact locations as these do not take into account prevailing wind direction, topography, landscape barriers such as tree lines, or ground cover that could impact the likelihood of contamination.

Regardless of the soil amendments utilized, each farm should have a soil amendment management plan as part of its food safety plan. This management plan should be supported by record keeping which documents:

- Type of soil amendment
- Source of soil amendment

- Relevant treatment or handling procedures (composting process, temperature monitoring, analysis, etc.)
- Application dates, rates, and fields where it was applied
- Set back distances or barriers between application areas and sensitive areas such as surface waterways

Production Water

Water quality is important because contaminated water can carry foodborne pathogens such as *E. coli* O157:H7, *Salmonella*, *Cryptosporidium*, and *Cyclospora* and transmit them to fresh fruits and vegetables (Beuchat and Ryu, 1997; Thurston-Enriquez et al, 2002; Steel and Odumeru, 2004). As mentioned earlier, there are no federal standards for irrigation water quality. In the absence of a federal standard, some industry organizations have adopted US EPA recreation water quality standards or other water quality benchmarks (USEPA, 1986; FDACS, 2007; CSFSGPHLLG, 2010). Establishing water quality standards is a difficult task particularly when surface water is being used for food production since some level of contamination is expected in water sources open to the environment. Well water is not an unlimited natural resource and it is critically important that decisions about limiting the use of surface water for the production of food crops take into consideration the actual risks of human illnesses and the limited supply of water alternatives (Bihn and Gravani, 2006).

When developing a production water management plan, there are three specific areas that should be thoroughly reviewed; water sources, methods of application, and timing of applications. Understanding these three management areas, assessing risks, and implementing practices to reduce identified risks is a logical approach to managing water use in the field.

Water Sources

Water used during production can come from many different sources such as municipalities including drinking and reclaimed water, wells, and surface water sources including rivers, streams and irrigation ponds. Usually, municipal drinking water is the safest and provides the lowest risk to produce safety, as it is treated and regularly monitored through testing by municipalities because it is intended as drinking water. Some municipalities have very old water lines that may be disrupted in places throughout the distribution system. Municipal drinking water sources used for the production of fresh produce should be tested at least once a year to verify the quality at the point of use. Test results should be reviewed and kept on file as part of good recording keeping practices.

Due to high water demand and low water availability, some municipalities provide reclaimed water for agricultural use. Reclaimed water is usually treated but not to the same extent as drinking (potable) water, so it can still contain human pathogens (Sadovski et al, 1978; Bastos and Mara, 1995; Oron et al, 2010). Treatment of reclaimed water may vary by municipality and location, so the quality of reclaimed water supplied may not be consistent. Understanding the risks associated with using reclaimed water are critical to choosing water testing strategies and application methods that allow for proper monitoring and risk reduction.

Ground water accessed through wells can be a source of water during fresh produce production. To maintain the safety of the water, wells should be properly constructed, capped, and well maintained. Well recharge areas should be kept free from livestock or any other things that could contaminate the ground water. Though wells should be a safe source of water, there is data from well-water surveys that indicates contamination of ground water by microbial human pathogens including enteric viruses can and does occur (Gerba and Smith, 2005). This

contamination can result from flooding or run-off from adjacent areas such as sewage treatment facilities or manure lagoons and may be impacted by soil type as well as hydrogeologic characteristics of the area. Suslow (2010) states that some wells became contaminated because of run-off into abandoned wells or uncapped bore-holes in close proximity to the well of interest, so simply maintaining and protecting the well that is the source may not be enough to protect the ground water source.

To monitor the quality of water, wells should be tested at least once a year to verify the microbiological quality of the water, and more often if there is reason for concern. Identifying other wells in the area whether in use or abandoned may also be valuable to understanding water quality issues on produce farms. If the water is used for drinking, the water should be tested for the presence/absence of total coliforms and nitrates/nitrites to ensure it is safe for human consumption as well as agricultural use. As always, test results should be reviewed and kept on file.

Surface water is likely to be of lower quality water than municipal and well water because it is open to the environment and most vulnerable to external contamination sources including run-off, wildlife, and livestock (Thurston-Enriquez et al, 2002; Hutchison et al, 2008). Monitoring the quality of surface water used in the production of fresh produce is important because it could impact produce safety. Conducting sanitary surveys of all surface water sources and regularly testing the water sources throughout the production season are two ways to implement a water monitoring program. A sanitary survey should include assessing upstream activities, reviewing land topography, evaluating feral animal activity, and visiting the water source to identify sources of potential contamination. All sanitary surveys should be documented and kept on file with other record keeping sheets.

All surface water sources used during production should be tested for quantified generic *E. coli* throughout the production season. The laboratory should be asked to use an analysis method that provides a quantitative result instead of an absence-or-presence test. Surface water testing allows growers to establish a baseline of expected water quality for their water sources and to determine if their current water quality meets the standards of buyers or commodity groups. If water test results indicate higher levels of *E. coli* than the maximum level set by the grower, growers need to take some action to mitigate the risk. These actions could include modifying water application practices, treating water, or using alternative sources of water. All actions should be documented and kept on file. A much more expansive discussion of surface water testing is present in Chapter 3 of this dissertation.

Method of Application

Methods of water application vary by farm size, crop, and region. Production water quality is most important when water is applied directly to the edible portion of the plant such as in overhead irrigation, the application of topical protective sprays, frost protection, and cooling. Research has shown that pathogens present in poor-quality water can persist in pesticide mixes, so only drinking water or water that is the microbial equivalent of drinking water should be used to mix topical sprays (Guan et al, 2001; Sathyanarayanan and Ortega, 2004). This importance of good water quality increases as the plants near harvest because there is less opportunity for UV solarization, desiccation, and other environmental factors to reduce microbial pathogens that may be present in the water.

Drip irrigation or other types of irrigation that deliver water directly to the root line represent the lowest risk irrigation method. There are other benefits to drip irrigation such as maximizing water use efficiency, improving yield, and keeping water off of the plant to reduce

plant pathogens. Installing drip irrigation in some production systems is not feasible, but it is one option for reducing microbial risks for those with poor quality water.

Timing of Application

The timing of the irrigation or spray application impacts safety because human pathogens can survive over time on the plant and in the soil providing a pathogen reservoir (Aruscavage et al, 2006; Wood et al, 2010). Overhead irrigation applied at planting or early in plant development represents less of a risk because it does not contact the edible portion of the plant. Recommendations in the scientific literature regarding the pre-harvest application of irrigation water applied overhead from surface water sources that promote acceptable risk reduction vary from days to several weeks even if the same microorganism is being studied (Hutchison et al, 2008; Wood et al, 2010). Applying the irrigation water in the morning to promote exposure to the sun and drying of the crop will promote the reduction of microbial populations on the plant (Steele and Odumeru, 2004). Managing water applications during the day and extending the pre-harvest interval are water management practices that can be implemented to reduce risks to fresh produce.

Preventing Backflow

Regardless of the water source and application method, it is important to inspect irrigation lines, spray equipment, and source water pipes to make sure they are equipped with backflow prevention devices. Backflow is the reversal of flow in a piping system that is opposite to the normal flow. Backflow can lead to unclean water contaminating clean water. All lines should be equipped with backflow prevention valves, and when filling from a hose or pipe, an air gap should always be maintained to prevent backflow. Lines from a well that feed into an

irrigation pond should not be lower than the overflow pipes since this could permit pond water to back up into the well.

In summary, water quality is most important when it comes into direct contact with the edible portion of a crop close to or at harvest. To reduce microbial risks associated with production water, vegetable growers should:

- Test all water sources for quantified generic *E. coli*
- Keep all tests results and file them with other produce safety records
- Conduct a sanitary survey of surface water sources
- Maintain all wells and inspect casing for cracks
- Mix topical protective sprays with water that is microbial equivalent to drinking water
- Understand risk associated with different irrigation methods
- Time the application of overhead irrigation to minimize risks

Wildlife

Wild animals such as deer, birds, and feral pigs are quite resourceful at gaining entry into produce fields and are commonly found in areas adjacent to fresh fruit and vegetable production. Wildlife is a concern because the animals can contaminate fields and surface water sources. They are known carriers of foodborne pathogenic microorganisms such as *Salmonella* and *E. coli* O157:H7 and wildlife species are very difficult to control due to their strength, agility, and numbers (Sargeant, 1999; Smith et al, 2002; Jay et al, 2007). If wildlife is identified as a problem, growers must actively pursue a solution to this problem.

It is the responsibility of growers to monitor wildlife activity, surface water sources and produce fields where animals may be present. If animal activity is confirmed in fruit and

vegetable fields through the presence of fecal material or commodity destruction due to mass animal movement paths, these areas should not be harvested due to the risk of contamination. Growers can utilize fencing or obtain nuisance permits issued by state agencies that allow for controlling wildlife that pose a danger to agricultural production.

Farming takes place within the natural environment and in some locations, conflicts between conservation and produce safety are becoming quite severe. Riparian habitat is being destroyed and growers are discontinuing their participation in conservation programs that promote clean water and wildlife habitat because buyers and food safety auditors are requiring them to have bare ground surrounding production fields (Beretti and Stuart, 2008). Although wildlife may pose produce safety risks, these risks need to be balanced with risks to the environment. Clean water, habitat conservation, and ecological diversity are all very important to a healthy natural environment that is capable of sustaining human life and agricultural production. Co-managing food safety practices and environmental programs may be challenging but takes into account the importance of both needs.

Postharvest Water

Any water applied to produce at harvest or during postharvest handling must be the microbial equivalent of drinking water. This includes water used to make top ice or water used to fill flumes or dump tanks. Using poor quality water at this point in produce production can result in contamination with foodborne pathogens or lead to postharvest decay.

There are many uses for postharvest water in the production of fresh produce such as cooling, moving commodities, washing, and waxing. There are several risks that should be considered when developing a postharvest water management plan, particularly if the water is recirculated or used in flumes, dump tanks, or other congregational water settings. Disinfection

of water is most critical if the water is recirculated or used in a congregational manner because one contaminated fruit or vegetable could contaminate the water and result in widespread contamination of other pieces in the batch. In a single event, an entire load could become contaminated if proper disinfection is not present. Water disinfection and temperature of the water in relationship to pulp temperature and depth of the dump tank are also important to prevent infiltration (Bartz and Showalter, 1981; Bartz, 1982; Zhuang et al, 1995). Risks related to postharvest water use can be managed if the risks are understood and identified.

Postharvest Water Sanitation

A critical aspect of managing postharvest water quality is disinfecting or sanitizing water, particularly if it is used in flumes, dump tanks, or recirculated in the system. Disinfectant levels in the postharvest water should be monitored to ensure they are at sufficient levels to limit both human foodborne and plant pathogens. Many fresh produce operations use sensors to determine the oxidation-reduction potential (ORP) status of their water. Maintaining an ORP between 650 and 700 millivolts (mV) will eliminate pathogenic bacteria as well as spoilage organisms (Suslow, 2004). ORP sensors can be combined with automatic injection systems that administer the disinfectant of choice directly to the postharvest water when the ORP drops below the set limit. There also are hand held ORP sensors and chemical kits that can be used to monitor disinfectant levels. All water monitoring protocols should be outlined in a Standard Operating Procedure (SOP) and documented as part of the farm or packinghouse food safety plan. It is important to remember, postharvest water affects both the safety and quality of the fresh produce it contacts.

There are many sanitizers that can be used to achieve this process, but chlorine is the most widely used due to its availability and affordability. When using chlorine, it is important to

monitor the levels of free chlorine since hypochlorous acid (HOCL) is the form of chlorine that kills bacteria and other disease-causing organisms. The presence of HOCL is pH dependent, so to achieve 80-95% free chlorine concentration the pH should be maintained between pH 6.5 and 7.0 (Suslow, 1997; Suslow, 2001; CCC, 2002). However, chlorine is less effective against protozoan foodborne parasites.

Parasites such as *Giardia lamblia*, *Cyclospora* spp., and *Cryptosporidium parvum* may be less susceptible to chlorine, particularly in waters with high organic load such as flumes and dump tanks (Jarroll et al, 1981; Leahy et al, 1987; Korich et al, 1990; Fayer, 1995; Carpenter et al, 1999). Of significant concern to the fresh produce industry is *Cyclospora* because it has been responsible for many produce associated foodborne illness outbreaks and very little is known about how this organism contaminates fresh produce (Veirk, 2008). It is also difficult to culture *Cyclospora* in the laboratory, making it difficult to study (Quintero-Betancourt et al, 2002). If the risk of contamination by protozoan parasites exists for the commodity of interest, then consider treating postharvest water with peroxyacetic acid, ozonation or high-intensity ultraviolet (UV) irradiation, rather than chlorine should be considered (Suslow, 2004).

Zhuang et al (1995) recommended that tomato packinghouses maintain dump tank chlorine levels at 200 ppm free chlorine and the dump tank water at a temperature higher than tomato pulp temperature. Other sanitizers such as acidified sodium chlorite, peroxyacetic acid, and gaseous chlorine dioxide may be more effective at reducing contamination on produce, so it is critical to review water disinfectant options and choose the one that is most appropriate for the operation and the commodities it handles (Yuk et al, 2006). Zhuang et al (1995) also suggested that tomatoes be stored at 10°C until they are ripened. Maintaining the cold chain is important because it reduces risks by preventing growth of pathogens such as *Salmonella* Typhimurium

and *E. coli* O157:H7 that can persist on commodities such as tomatoes, bell peppers, and cantaloupes at cold storage temperatures (5°C and 10°C) (Zhuang et al, 1995; Alvarado-Casillas et al, 2007). Maintaining the cold chain reduces risks but does not eliminate all risk because any remaining bacteria can then be transferred into the fruit flesh during cutting and processing (Selma et al, 2008)

Commodity characteristics, the type of foodborne pathogens, postharvest handling practices, water temperature, and water disinfection all impact fresh produce safety, so it is important to review farm and packinghouse practices with these factors in mind. Whenever dump tanks, flumes, or other water immersion steps are part of postharvest handling, water and pulp temperatures should be monitored to ensure the water is warmer than the fruit to prevent infiltration. Water disinfectant levels also should be monitored to ensure foodborne pathogens and spoilage microorganisms are controlled in postharvest water.

Infiltration

Research related to dump tank and flume water infiltration of tomatoes began after Bartz (1980) noted that a shipment of fresh market tomatoes was rejected at the receiving point due to decay. Virtually all of the lesions in a representative box of this shipment had begun inside the fruit. Subsequently, Bartz and Showalter (1981) determined that fruit physiology (fresh stem scars) and fruit temperature (warmer than dump tank/flume water) led to a significant infiltration of fruit with water. Subsequently, hydrostatic forces (increased pressure due to immersion deeper down in the tank) were linked with water absorption by fruit. Studies conducted with *Salmonella* Montevideo have verified that pathogens can enter fresh produce through water used during postharvest activities (Zhuang et al, 1995). Certain fruits and vegetables have been

identified as being susceptible to infiltration including mangoes, tomatoes, peppers, and melons (personal communication, Michelle Smith).

Postharvest handling of melons, tomatoes, and peppers may include passage through a water flume or immersion in cold water (32°F) prior to processing for the fresh cut market. Figure 2 depicts the result after a cantaloupe was placed in a ziplock bag with water soluble methylene blue dye. The bag was then submerged in an ice bath. After the fruit had cooled, the dye was washed off. In Figure 1-2 the extensive penetration of the dye to the interior flesh is visible (Personal communication, Jerry Bartz). This dye also penetrated directly through the peel and through the netting on the outside of the melon. A similar process was applied to a tomato, with Figure 1-3 documenting the nigrosin dye penetration of the tomato stem scar. Additional infiltration studies using an aqueous cell-suspension of *Erwinia carotovora* resulted in soft rot contamination in the interior of the tomato fruit (personal communication, Jerry Bartz). These images provide visual confirmation of infiltration, but there are other factors that may adversely impact product safety and quality.

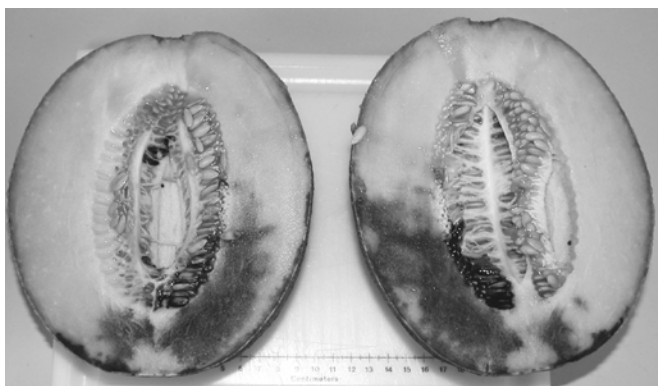


Figure 1-2. Cantaloupe infiltrated with methylene blue dye as a demonstration of postharvest handling risks.



Figure 1-3. Nigrosin dye penetration of the tomato stem scar.

Mangoes, melons, tomatoes, and peppers have unique phenotypic characteristics or handling requirements that may increase their susceptibility to infiltration. As a group, these

four commodities represent an opportunity to highlight the importance of understanding commodity specific attributes critical to implementing practices that minimize food safety risks. In the category of melons, cantaloupe is of particular concern because the surface netting creates areas where bacteria can attach and be protected from removal by wash tank waters or spray applied sanitizers (Alvarado-Casillas et al, 2007). In particular, *Salmonella* has been the cause of several cantaloupe associated foodborne illness outbreaks. In studies where cantaloupes were inoculated with a cocktail of foodborne pathogens containing *Salmonella* strains, *E. coli* (O157:H7 and non-O157:H7), and *Listeria monocytogenes*, *Salmonella* exhibited the strongest attachment (Ukuku and Fett, 2002; Vierk, 2008). This result suggests that bacterial attachment is not simply dictated by the surface of the commodity but is influenced by characteristics specific to the microorganisms or a synergistic effect between the commodity surface and the microorganism.

The surface of tomatoes and peppers are smoother, but may still have micro-structures and be susceptible to injury or abrasions. Viable *Salmonella* has been recovered from contaminated tomatoes even after submersion in a scale-model flume containing 150 mg/L free chlorine for two minutes (Felkey et al, 2006). Stem scars and puncture wounds were identified as areas most difficult to sanitize in both bell peppers and tomatoes (Felkey et al, 2006; Yuk et al, 2006). Once contamination occurs, it is difficult to remove and identify if it is not associated with rot or some other visual indicator that would result in it being culled.

A multistate outbreak of *Salmonella enterica* serotype Newport (SN) that resulted from the consumption of mangoes highlighted how handling practices combined with insufficient postharvest water management could result in internalization of pathogens. As a method of preventing the importation of the Mediterranean fruit fly into the United States, mangoes

received a hot water immersion treatment followed by cool water immersion treatment. This hot to cold water transition likely resulted in infiltration and internalization of water containing SN (Sivapalasingam et al, 2003). Postharvest water management failed to properly and consistently disinfect the cool water. Although the water was initially chlorinated, the levels were not monitored and the water changing schedule was not standardized to prevent microbial load build-up. In addition, pest control issues including the presence of toads, birds and bird feces were noted that were not monitored or addressed adjacent to the unenclosed dip tanks. This outbreak caused 78 individuals in 13 states to be infected with the outbreak strain of SN, resulting in two deaths and 15 hospitalizations (Sivapalasingam et al, 2003). This was the first reported outbreak of SN in mangoes but it led to the identification of a postharvest handling step that increased the food safety risks associated with this commodity. Identifying commodities that are at risk for infiltration by postharvest water and understanding the importance of postharvest water management as well as pest control are important for proper risk assessment and food safety practices implementation to reduce food safety risks to fresh produce.

Cleaning and Sanitation

Maintaining a clean operation covers a multitude of areas ranging from simply keeping fields free of debris to detailed Sanitation Standard Operating Procedures (SSOPs) for cleaning and sanitizing specific pieces of equipment. Starting broadly, the general organization and appearance of an operation should be considered. All food production facilities should be clean and organized, and fresh produce farms and packinghouses are no exception. There are certainly challenges that exist as anyone who has been in a functioning packinghouse on a rainy day can attest. Soil, plant debris, rotten fruits and vegetables, and used packing containers, are all present and need to be managed. This is why every farm and packinghouse should establish basic

cleaning and sanitation protocols such as sweeping packinghouse floors at the end of each day and follow procedures to assure a clean and organized operation. All trash should be removed from fields and packinghouses and deposited in secured dumpsters.

Beyond these basic behaviors, each farm owner or operator needs to evaluate their operation and determine the need for specific SSOPs. For instance, if harvesting fruits and vegetables requires the use of harvest aids such as knives, they should be cleaned and sanitized at appropriate times during and at the end of each day. The cleaning and sanitizing procedure should be detailed in an SSOP to ensure the process is done the same way each time and that it is effective. If harvest containers are reused, these too should be cleaned and sanitized on a scheduled basis. If the harvest bags cannot be sanitized due to the material of the bag, they should at least be cleaned of visible debris each day and stored in a clean location until the next use.

Harvest containers made of wood represent one challenge to cleaning and sanitizing during fruit and vegetable production. Wood is very porous and not easily cleaned or sanitized. If wooden packing or storage crates are currently in use, consider replacing them with durable plastic crates that are easily cleaned and sanitized as the wooden crates break and need to be replaced. If a packing facility is being renovated or a new packing facility is being built, it would be extremely valuable and prudent to review all building plans to ensure the principles of sanitary design are incorporated. Sanitary design principles address the design of space and equipment placement so that they can be easily and effectively cleaned and sanitized. Proper design results in significant savings in both time and money spent on human resources and chemicals. When updating equipment such as packing lines, wood or other porous materials should be replaced with stainless steel because it is easier to clean and sanitize and will better

withstand exposure to cleaning and sanitizing chemicals. In discussing the cleaning and sanitizing of equipment and facilities, the word soil refers to unwanted matter including field soil, plant material, and other unwanted material that could contaminate produce. Proper procedures for cleaning and sanitizing need to be used to reduce microorganisms to a safe level. The four steps involved in cleaning and sanitizing are review in Table 1-1. Identifying detergents and sanitizers that are appropriate for the type of soil that needs to be removed as well as the type of equipment being cleaned is also important. Chemical suppliers or extension educators can be a valuable resource by providing farm operators with information to assist them with choosing appropriate detergents and sanitizers.

Table 1-1. The four steps involved in cleaning and sanitizing food contact surfaces such as harvest aides, harvest containers, and packing lines.

1 Prerinse	The first step in the cleaning and sanitizing process is to pre-rinse surfaces to remove soil that may have accumulated, paying particular attention to cracks, crevices, and hard-to-reach areas. Pre-rinsing may require physical actions such as scraping and brushing to remove the soil.
2 Wash	Step 2 requires a thorough washing (cleaning) of the surface to disperse the soil in the detergent solution. All detergent (cleaner) should be mixed according to label directions and applied to the surface to break down the soil and all its components including fats, carbohydrates, and proteins. The chemical action of the detergent and the physical action of scrubbing will help to remove the soil.
3 Rinse	In step 3, the detergent solution containing the soil is rinsed away. This rinse step ensures that the surface is visibly free of soil and detergent solution.
4 Sanitize	In step 4, a sanitizer is applied to the surface as directed on the label. All sanitizers should be mixed according to label directions and tested with a simple test kit specific to the sanitizer being used to determine that the appropriate concentration (strength) has been achieved. Sanitizers reduce the level of spoilage and pathogenic microorganisms on the surface to safe levels. Step 1–3 must be done properly because if the surface is not clean, then the sanitizer quickly loses its effectiveness.

Pest Control in Packinghouses

Unlike fields, enclosed packinghouses have walls, doors, and windows that can be used to limit pest entry. Efforts to control pests in packinghouses should focus on four specific goals (Marriot and Gravani, 2006):

Preventing entry

Eliminating shelter

Eliminating food sources

Eradication

Preventing Entry

All doors should fit properly so that there are no gaps around the doors when they are closed. Rats can enter through a hole the size of a quarter and mice through a hole the width of a pencil. Flies and other pests can pass through even smaller openings. Door seals and screens should be in place and maintained to minimize entry opportunities. Packinghouse doors are often open throughout the day as loads are moved in and out, so efforts should be taken to restrict pest entry as much as possible by closing outside doors when not in use, or installing deterrents such as strip or air curtains.

Eliminating Shelter

Keeping the outside of the packinghouse well mowed and removing debris such as old pallets, boxes, and unused equipment will reduce shelter that could be used by pests. In the packinghouse, pallets should be stacked one foot or more away from the walls so that pest control measures such as mechanical traps can be used and monitored. Some packinghouses have ceiling cross bars that provide roosting areas for birds. If birds are a problem, netting can be used to cover the ceiling area to deter bird roosting. Removing harborage areas will deter pests and reduce risks associated with infestations.

Eliminating food sources

Employee areas such as locker rooms, break areas, and lunch rooms should be kept clean and organized. All food items should be properly stored in sealed containers to limit pest access to food. Unused seed should be stored in sealed containers away from the packing areas. Culled

fruits and vegetables should be removed from the packinghouse daily and not piled near the outside of the packinghouse. Food sources serve as an attractant to pests and allow them to persist in an area, so eliminating food sources is critical to any pest management program.

Eradication

Snap traps, glue boards, mechanical traps, and other eradication devices should be used to actively eliminate packinghouse pests and provide an opportunity to identify pests that are present. Never use poison bait that can be translocated by pests inside the packinghouse, as this puts commodities at risk for contamination. Poison bait stations can be used outside and around the perimeter of the packinghouse to control pests outside of the packinghouse. If using insect control lights, be certain the lights are not visible from the outside as this will attract insects inside the packinghouse. Place lights on the interior walls facing into the packinghouse to eliminate insects that are in the packinghouse without attracting new insects. There may be other eradication strategies that can be used to effectively control pest, but the important point of this discussion is to have a pest control system in place and monitor it for effectiveness.

All pest control measures should be outlined in the farm's food safety plan. Pest monitoring records should be kept noting a map of all traps in the facility, the date traps were checked, and any pests that were present. All actions taken to control pests also should be noted. If an outside pest control company is being used, request that they provide a detailed list of their findings so that this information can be kept on file and farm personnel can verify that they are controlling relevant pests.

Traceability and Recall

In the event of a foodborne illness outbreak, determining the origin of the outbreak is important for stopping the outbreak as well as determining its cause. Being able to track a food product through the food system is called traceability. Growers cannot necessarily be expected to trace their crops from farm to table because of the complex nature of commodity movement through fields, packinghouses, terminal markets, retail stores and homes, but growers do have a responsibility to have a traceability system in place that tracks the commodities they grow and distribute. Standard traceability programs focus on one step back and one step forward; where did the produce originate (field) and where did it go (buyer). If everyone in the food system could trace the produce one step back and one step forward, all produce items could be quickly traced from the consumer to their point of origin during foodborne illness investigations.

This topic of traceability is receiving significant attention in the fresh produce industry as well as in Congress. The Produce Marketing Association, United Fresh Produce Association, GS1 US, and the Canadian Produce Marketing Association have developed the Produce Traceability Initiative (PTI) with the goal of “achieving supply chain-wide adoption of electronic traceability of every case of produce by the year 2012” (PTI.org). PTI is just one example of how traceability is impacting the industry, but the new FDA Food Safety Modernization Act that was signed into law January 4, 2011 also contains traceability language and outlines for establishing pilot projects to develop effective means of tracking foods throughout the food system. Whether it is industry initiatives or congressional mandates, the need for traceability is clear. The pressure for growers to develop and maintain a traceability system is only going to increase. At some point in the near future, there may be standards for traceability systems. Until it is a requirement or until one traceability system is agreed upon, it is important for growers to develop

and implement a traceability system that works for their operation. Initial focus should be placed on identifying lots and integrating current management practices into an effective traceability system.

A “lot” is simply a defined and finite portion of a crop. A lot could be defined as individual loads that are sold, but a more useful lot definition would identify a particular harvest from a particular day from a particular field. In attempting to determine lot size, it may be beneficial to consider what would happen if a lot was recalled; the larger the lot, the larger the recall, and the larger the potential loss. On the other hand, identifying each pallet as a lot requires much more management and detailed traceability. Some harvest practices, such as those used by the potato industry, present significant challenges since most of the harvest is literally piled into one lot or just a few lots. Working with growers to identify steps to improve lot identification that are practical and feasible to implement is one area where food safety extension personnel can help the fresh produce industry.

Product labeling also is part of a traceability system. Each farm should develop a labeling system so that minimally, each lot is labeled with the farm name and relevant contact information. Ideally, each piece would be labeled so that it could be traced to the farm of origin, but at this time that is not practical to consider for most commodities. As of March 16, 2009, the Country of Origin Labeling (COOL) law became effective mandating that all fresh fruit and vegetable producers who directly or indirectly supply retailers identify the country of origin of their commodities either on the product, on the shipping container, or in the documents that accompany the shipment. To be identified as a US product, the commodity must be harvested in the US (<http://www.ams.usda.gov/AMSV1.0/cool>, accessed 6/30/2009).

This represents the first legal requirement for fresh produce labeling at the farm level and was expressly implemented to provide consumers with more information about the origin of their food. With continued advances in communication and labeling, it is becoming financially and technologically feasible to identify and trace individual pieces from the farm to the table. Each farm or operation needs to determine how to best define parameters important for traceability, develop a traceability system, and test the system.

Each farm should develop a Recall Plan to support its traceability system. Figure 1-4 provides an outline of information that should be contained in a Recall Plan. The Recall Plan will require farm personnel to gather and organize important contact information for buyers and other farm resources. The time and energy invested into this activity will prove valuable during any mock recalls the farm conducts as well as in the development of a crisis management plan discussed later in this chapter.

One item on the Recall Plan Outline suggests identifying a specific person on the farm to be the media contact. Media training is not always an obvious asset to many fresh produce growers. It would be valuable to have someone on the farm that has media training since a recall will very often attract media attention. How the recall is portrayed in the media can directly impact the farm either positively or negatively, so for the best outcome possible all farms should consider having someone trained to deal with the media.

A Recall Plan should include:

- Names and contact information of key employees that are members of the recall team
- A specific person who will be the media contact
- Processes for notifying the public and regulatory agencies
- Procedures for implementing the recall
- Strategies for handling recalled produce
- Methods for verifying recall plan effectiveness, including removal of product from the marketplace
- Means of communicating with the customer
- List of critical farm operations that must be maintained during a recall
- List of resources, including testing labs, available to the farm in the event of a recall
- Current phone and fax numbers and email addresses of
 - Key farm management staff
 - Produce buyers and distributors
 - Federal and state regulatory agencies
 - Description of produce and container sizes you market
- Description of how you label and identify lots (units). Lot identification/labels should be able to link each individual lot to the:
 - Grower(s)
 - Field (location)
 - Date harvested or date received if co-packing
 - Individuals involved in harvesting
 - Total number of packages in the lot

Figure 1-4. Recall plans will help ensure recalled product is removed from the market efficiently and effectively. This outline provides guidance for the development of a recall plan.

Mock Recall

An effective way to test a traceability system and recall plan is to conduct a mock recall. In a mock recall, a farm representative contacts one of its past buyers in an attempt to locate a particular lot and determine how much of the lot has been sold and how much of the lot is still in stock. The farm representative should be able to identify where the lot was grown and when it was harvested, packed, and shipped. The mock recall should be documented and any problems should be identified so that the traceability system can be modified and improved. The mock recall is also an opportunity to review the recall plan and update contact information.

Crisis Management

Many things can result in crises on farms and in packinghouses such as chemical spills, tractor accidents, foodborne illness outbreaks associated with grown commodities, or the injury or death of a key farm employee. Every farm should have a crisis management plan as part of their farm produce safety plan. The recall plan outlined in Figure 4 provides a good summary for the development of a crisis management plan. Much of the information may be the same, particularly in smaller operations. The crisis management plan should identify a crisis management team and list their contact information including cell phones and home phones so they can be reached immediately. In small operations, the team is likely to be very small and may require the inclusion of individuals who do not work for the farm. In this case, it is important that everyone know they are on the team and that they understand what their role will be should a crisis occur.

The crisis management plan should identify buyers and any individuals who conduct business with the farm so they can be easily contacted if production is interrupted. Any resources that would be of value to the farm during a crisis should also be listed such as insurance company representatives, lawyers, and grower organization contacts. Developing a crisis management plan and assigning responsibilities is best done before a crisis. Those working directly with fresh fruit and vegetable growers and packers should encourage the development and implementation of a crisis management plan.

Third Party Audits

Third party audit verification is an attempt to guarantee produce safety practices such as GAPs have been implemented. These audits are conducted by a third party that the grower or buyer hires to conduct the audit. They are usually announced and take several hours to several

days to complete depending on the size of the operation. The rest of the time there is no auditor on site monitoring the implementation of the produce safety plan. Some audit companies are introducing unannounced follow up visits in an attempt to verify practices in a true day to day setting, not when operations have had weeks to prepare. This increases the costs of the audits since it requires an additional visit, but it is intended to audit how actual practices are implemented on a day to day basis.

An important point regarding audits is that merely passing an audit does not guarantee the operation has implemented a food safety plan or that it prioritizes food safety. As an example, on March 27, 2008, Peanut Corporation of America (PCA) in Blakely, GA received a “Superior” rating from a third party audit company (PCA, 2008). On November 25, 2008, an epidemiologic assessment began of a growing cluster of *Salmonella* serotype Typhimurium isolates that would later be linked to individuals that had eaten peanut products from PCA (CDC, 2009). On January 28, 2009, PCA announced it was voluntarily recalling all peanuts and peanut products processed in its Blakely, Georgia facility since January 1, 2007, because they have the potential to be contaminated with *Salmonella* (PCA, 2009). This highlights the fact that audits are not fool proof and that food safety needs to be built into all operations and practiced daily.

Third party audits for fresh produce farms are offered by many companies including NSF Davis Fresh, American Institute of Baking (AIB), Primuslabs.com and the United States Department of Agriculture's Agricultural Marketing Service to name a few. An unintended consequence of having access to several different third party audits is audit fatigue. Not all growers are required to have a third party audit, but some buyers will only accept third party audits from a company (or companies) that they designate. If growers sell to multiple buyers who each want a different audit company to conduct the audit, the grower is forced into having

several different audits to meet all the buyers requirements resulting in audit fatigue. This is not only difficult to manage but very expensive. In 2009, United Fresh Produce Association organized a Steering Committee and Technical Working Group to spearhead an attempt to develop a harmonized audit document. They have completed two harmonized documents entitled Field Operations and Harvesting, and Post-Harvest Operations that are available at http://www.unitedfresh.org/newsviews/gap_harmonization. It is not clear how widespread the adoption of these standards will become but it is a clear indication that the produce industry is interested in having comprehensive standardized audit requirements.

Food Safety Everyday

Ideally, each farm and packinghouse would have a written and implemented food safety plan that is based on a risk assessment of their operation and of the commodities they produce. Each operation and each commodity they grow have different risks that need to be addressed depending on how they grow, harvest, pack, transport, and market the commodities (Dallaire et al, 2006; Ailes et al, 2008). The need for produce safety in fruit and vegetable production cannot be disputed as indicated by the many produce-associated foodborne illness outbreaks that have occurred (Vierk, 2008). In a survey of fruits and vegetables available in retail markets over a two-year period, *Salmonella* was found on eggplant, sweet potato, peppers, tomatoes, cucumbers, butternut squash, green onions, and carrots (Wells and Butterfield, 1999). The incidence of *Salmonella* was low overall but was higher if the produce had bacterial soft rot evident whereas mechanical injury and fungal rots did not increase incidence. The significance of this is that many commodities were presumptive positives for *Salmonella* and certain attributes such as bacterial soft rot increased the incidence but was not the only factor. Science has yet to clearly define all the factors at work, but given the risk to the produce industry and to

the viability of individual farms, all produce growers even if the commodities they produce have never been involved in a produce-associated foodborne illness outbreak should be concerned about produce safety.

It may seem easy to demand produce safety, but field production and postharvest handling provide many challenges. Many small operations are heavy on the workload and light on the human resources. Fresh produce growers are more familiar with quality issues and marketing issues than produce safety issues. For better or worse, produce safety has now become a marketing issue, as more and more buyers are demanding produce safety plans and audits to verify produce safety practices. In addition, the FDA plans to release a draft produce safety regulation by the end of 2011. The FDA Food Safety Modernization Act included an amendment exempting farms that sell under \$500,000 gross sales of fresh fruits and vegetables from produce safety regulations. Even if these farms are exempt from the regulation, they are not exempt from the market place and up until now, implementation of produce food safety practices has been market driven. It seems likely this will continue, so even if growers do not have to follow the law they will still have to meet buyers' demands for food safety practices. All fresh produce growers need to proactively develop a food safety plan to ensure not only the well being of consumers, but also the sustainability of their farms.

The research contained in this dissertation will explore several different aspects of produce food safety. First, a survey of fresh fruit and vegetable producers in New York will explore water sources, water application methods, current water testing practices, and current evaluation practices of adjacent land being used during the production of fresh produce. This survey also asked growers to identify if their buyers are asking about their food safety practices. Additional chapters will focus on the quality of surface water sources used on selected farms

throughout New York State. Initial analysis of water sources included the determination of quantified generic *E. coli*, turbidity, pH, and specific conductance over two growing seasons. Some surface water sources were further analyzed for the presence of *Salmonella* late in the second season. The significance of combining a grower survey with surface water quality analysis is to draw the very important line between the ultimate end user of produce safety related research. If growers are unaware of the research or fail to implement practices based on science that are indicated to reduce risks, then the goal of risk reduction in the production of fresh produce is lost. The other critical link is getting the science based recommendations to the grower which has traditionally been done through extension at Land-Grant Universities throughout the United States. This mission seems to be changing as funding at universities is reduced and priorities reorganized, but it is in the spirit of generating good data to develop effective risk reducing practices that growers understand and can implement that this dissertation is offered.

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Chapter Two

Use of Surface Water in the Production of Fresh Fruits and Vegetables: A Survey of Fresh Produce Growers and their Water Management Practices

Abstract

Surface water is an important natural resource and is critical to the production of fresh fruits and vegetables. It also represents a potential microbial hazard that could impact the safety of fresh fruits and vegetables when used during production, particularly if it is applied directly to the edible portion of the plant during irrigation, frost protection or the application of protective topical sprays. Currently, there are no national surface water standards for water used in the production of fresh produce, though with the increase of produce-associated foodborne illness outbreaks, several commodity groups have adopted standards based on the Environmental Protection Agency's Ambient Water Quality Standards for recreational water use. A survey was conducted in the winter of 2008-2009 to assess current surface water management practices by fruit and vegetable growers in New York State. This survey was developed to better understand current irrigation water sources, methods used to apply the water, testing frequencies, and adjacent land assessment to identify risks that may exist. New York growers are frequently utilizing surface water sources and applying it overhead to grow a wide range of fresh produce commodities but testing for microbial quality indicators and adjacent land assessment for risks are not common practices.

Introduction

The production of fresh fruits and vegetables is dependent on many environmental variables such as temperature, sunlight, rainfall, and soil type. Fresh produce growers can and

do modify the growing environment to ensure crop quality and productivity. The farm environment provides many opportunities for contamination to occur. Soil amendments (i.e., manure, compost and compost teas), direct contact with wildlife, airborne deposition from off-farm activities such as cattle/dairy and manure/composting operations, and water (irrigation or flooding/runoff from adjacent land) all represent potential mechanisms for contamination by foodborne pathogens such as *E. coli* O157:H7 and *Salmonella* (Beuchat and Ryu, 1997; Beuchat, 2002; Aruscavage et al, 2006; Brandl, 2006). Much fresh produce is consumed raw and therefore does not receive any treatment step that would kill foodborne pathogens that may have contaminated the fresh produce during production and packing. Over the past several decades, the consumption of fresh fruits and vegetables that were contaminated with foodborne pathogens has resulted in foodborne illness outbreaks (USFDA, 2001; Sivapalasingham et al, 2004; Vierk, 2008). Four specific human pathogens including *E. coli* O157:H7, *Salmonella*, *Cyclospora*, and Hepatitis A have accounted for 96% of the outbreaks and 95% of the illnesses in reported produce related outbreaks from 1996-2007 (Vierk, 2008). Contamination of fresh fruits and vegetables with foodborne pathogens can occur at any point in the supply chain and depending on the commodity, is difficult, if not impossible to remove because of phenotypic and physiological traits (Beuchat and Ryu, 1997; Gagliardi et al, 2003; Stine et al, 2005; Doyle and Erickson, 2008).

Water is an important natural resource, is critical to the production of fresh fruits and vegetables, and is often used to enhance growing conditions. The application of irrigation water, water for frost protection, and topical protective sprays are just a few of the practices that growers utilize during the growing season to promote crop growth and productivity. The water used for these practices can come from multiple sources including surface water, well water and municipal water. These multiple sources of water can be applied in several different ways such

as through drip tape, overhead sprinklers and spray machinery, and the type of delivery will often determine the volume of water needed as well as the pressure.

Many farmers use surface water including streams, ponds, and lakes as their water source to irrigate and apply protective sprays to fresh produce. In many instances, little may be known about the quality of this water because water testing is not implemented and there are not clear, consistent, universally accepted recommendations to guide farmers through the water monitoring process. The concern about the quality of water used to grow fresh produce is directly related to the concern about the foodborne illness risks it may represent. Safety concerns arise when water is applied directly to the edible portion of the crop thereby depositing any contamination that may be in the water directly onto the crop. Many foodborne pathogens of concern are carried in water including *Salmonella enterica*, shiga-toxin producing *E. coli*, *Campylobacter jejuni*, and *Cryptosporidium* and could be spread through irrigation and topical spray applications especially when they are applied directly to the edible portion of the crop (Cornell 2010, Mootian et al, 2009).

Water applied to fruits and vegetables not only impacts the safety of the crop, but can also impact the safety and water quality in local watersheds. The impact to local watersheds occurs through both the use (removal of water) and the return (application) of the water to the crop land (environment). Growers not only have to manage the safety of the crops they produce but also must manage their environmental impact to both land and water.

The safety of fresh produce and environmental impacts should be a concern to all fresh produce growers because of the ramifications to their customers as well as to the financial viability of their operations from both a liability and economics standpoint. Following well publicized produce associated foodborne illness outbreaks, many retail buyers now require fresh produce growers to test their irrigation water prior to use and develop a water management plan

to reduce food safety risks. In addition to the impact on markets, foodborne illness outbreaks have resulted in commodity groups adopting new practices and requirements in an attempt to control risks (FDACS, 2007; CSFSGPHLLG, 2010). Currently there are no federal irrigation water quality standards, but the Commodity Specific Food Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens adopted the Environmental Protection Agency Ambient Water Quality Standards as the irrigation standards required during production (USEPA, 1986; CSFSGPHLLG, 2010). The use of these standards is interesting because they were developed for recreational waters not production agriculture. Due to the lack of national irrigation water quality standards, these EPA standards are the current benchmark used by several fresh produce commodity groups. In addition to the produce industry movement toward irrigation water standards, the Food and Drug Administration has stated that they will be releasing a produce safety rule in the next 12 months and it is reasonable to assume the quality of irrigation water may warrant the inclusion of specific language regarding irrigation water quality standards.

Depending on the year, New York is either first or second in cabbage production, second in apple production, and eighth in Strawberry production in the nation. In addition to these crops, New York farms produce a diverse array of fresh fruits and vegetables that run from asparagus to zucchini. These crops are marketed locally, statewide, nationally, and internationally. The safety of New York grown produce impacts many consumers every day.

This survey was developed to better understand current irrigation water sources and water management practices including delivery methods being used by fresh produce growers in New York. Questions were designed to assess if testing of water sources was common and what types of water tests were being conducted as well as the frequency of testing. Participants were

also asked about environmental factors near their farms that could impact the safety of their water sources and the produce they grow including adjacent land use and manure application practices. Additional questions were targeted to determine if fresh produce buyers are inquiring about food safety practices and if New York growers are actively engaged in developing farm food safety plans.

The resulting data allows for the assessment of current water management practices by New York growers to help determine how the industry may be affected if federal standards are mandated regarding irrigation water delivery or irrigation water quality. Although this survey was only conducted in New York, the information obtained is relevant to growers beyond New York because many of the commodities grown and management practices utilized are common in other states. Results from the survey will assist in the development of educational materials and extension training aimed at encouraging risk assessment and the implementation of food safety practices on fresh produce farms to reduce risks and meet market demands for food safety.

Materials and Methods

A survey containing 18 questions including basic demographic information was developed for fresh fruit and vegetable farmers and distributed throughout New York State (Appendix 2-A). The survey contained questions to determine current irrigation water uses, sources, and management practices, as well as questions pertaining to commodities grown and possible risk factors related to adjacent properties. In addition, questions to help determine if buyer demand for food safety programs exist, and if so, what growers are doing to meet this demand were included. Participants were asked to provide demographic data including age, county where they reside, and size of their entire farm. This project and the survey were

reviewed and approved by the Institutional Review Board for Human Participants at Cornell University.

The survey was produced in both an electronic and paper format to encourage participation. Growers were provided access to the survey through direct mailings, e-mail listserves and monthly newsletters written by extension educators throughout the state of New York including *Muck and Mineral*, *VegEdge*, *Fruit News*, and *New York Berry News*. Circulation of these newsletters accounted for approximately 1,183 homes. Some of the newsletters contained both a paper survey and the link to the electronic survey, while others just included the electronic link to the survey. A mailing list that contained 197 addresses of growers or farm operations was sent a paper survey with a stamped, addressed return envelope, as well as the link to the electronic survey. Responses were analyzed to determine sources of irrigation water, methods of application, use of water testing, commodities produced using surface water irrigation, average acreage irrigated, as well as adjacent land uses that might represent risks to surface water sources or to fresh produce fields.

Results

Demographics

A total of eighty four surveys were completed and submitted by farmers. Seventy five paper surveys were submitted through the United States Postal Service and nine surveys were completed online and submitted through the Checkbox survey collection site. Responses from farmers living in at least fifteen different counties throughout New York including Dutchess, Erie, Genesee, Monroe, Niagara, Ontario, Orange, Orleans, Schuyler, Steuben, Suffolk, Tioga, Tompkins, Wayne, and Yates were received (Figure 2-1). Suffolk and Niagara counties had the

highest number of participants with both having 12 respondents who participated and identified their county. Fifteen respondents chose not to provide their county. Respondents were 20 to over 70 years of age, with 31% (26 of 84 respondents) being in the 50-59 age range, accounting for the highest percentage of respondents (Figure 2-2). This supports 2007 census data from the National Agricultural Statistics Service that found the average age of principal farm operators in New York to be 56 years of age (USDA-NASS, 2007).

Participating farmers indicated their farm sizes from one acre to more than 1,000 acres. The option of less than one acre was provided but was never selected. The largest response rate was from farmers who indicated farm size as 11-50 acres providing 20 of 84 responses (24%) (Figure 2-3). The average farm size in New York is 195 acres according to the 2007 census data from the National Agricultural Statistics Service (USDA-NASS, 2007). In this survey, 17 respondents (20%) reported having farms that ranged from 101-200 acres.

Distribution rates among counties, age, and farm size including commodities grown was very good, indicating that a diverse set of state farmers participated in the survey. These farmers represented diverse sized farms growing a range of commodities. Given the size of farms and commodities produced, it is likely these farms represent variable marketing practices including direct marketing to consumers and wholesaling, those this cannot be confirmed through this data set because this specific information was not collected, Two specific production regions of New York were represented in the survey, namely western and southeastern NY.

Surveyed Counties

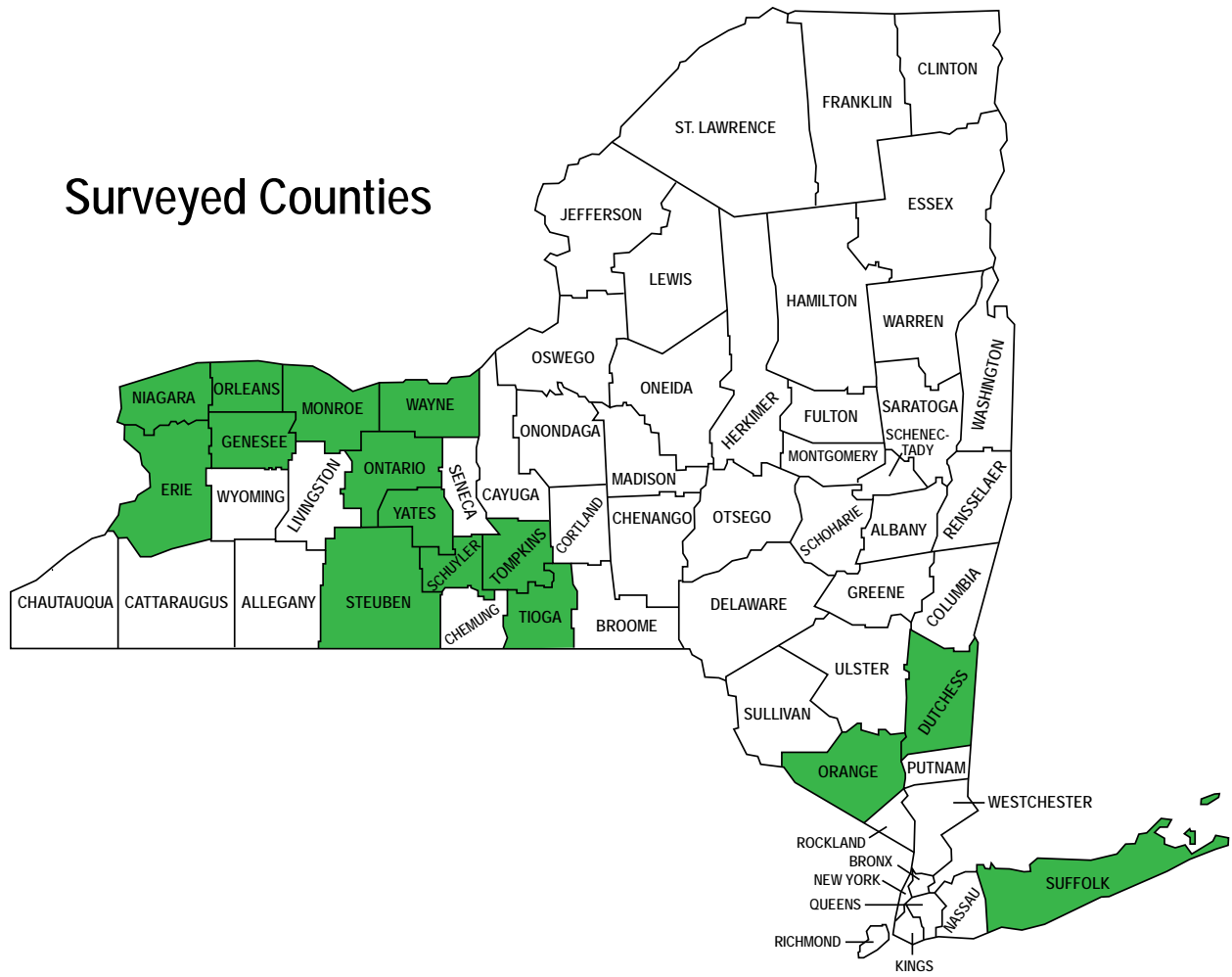


Figure 2-1. Counties with individuals who participated in the survey are colored green.

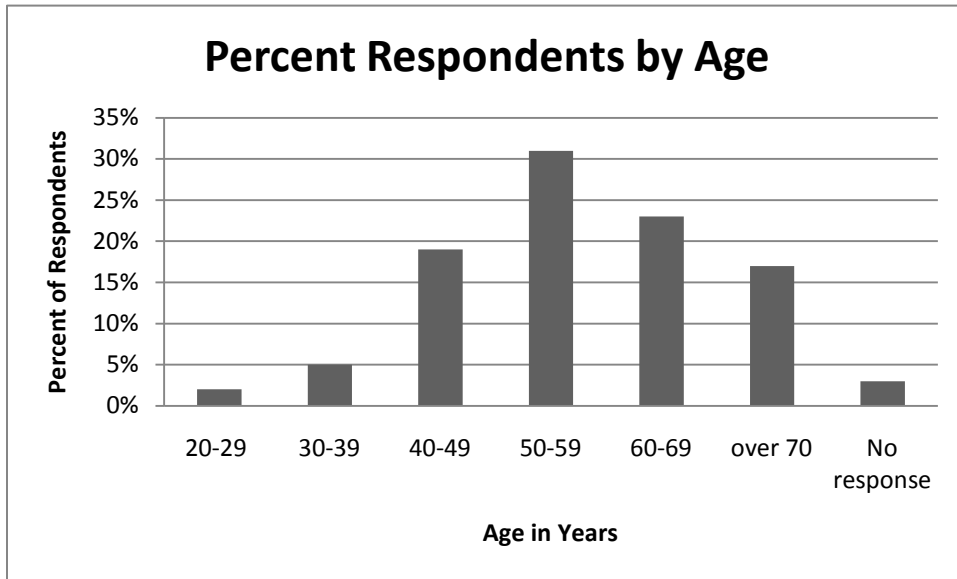


Figure 2-2. Growers in the range of 50-59 years of age accounted for the highest level of participation reflecting the average age of 56 for growers in New York.

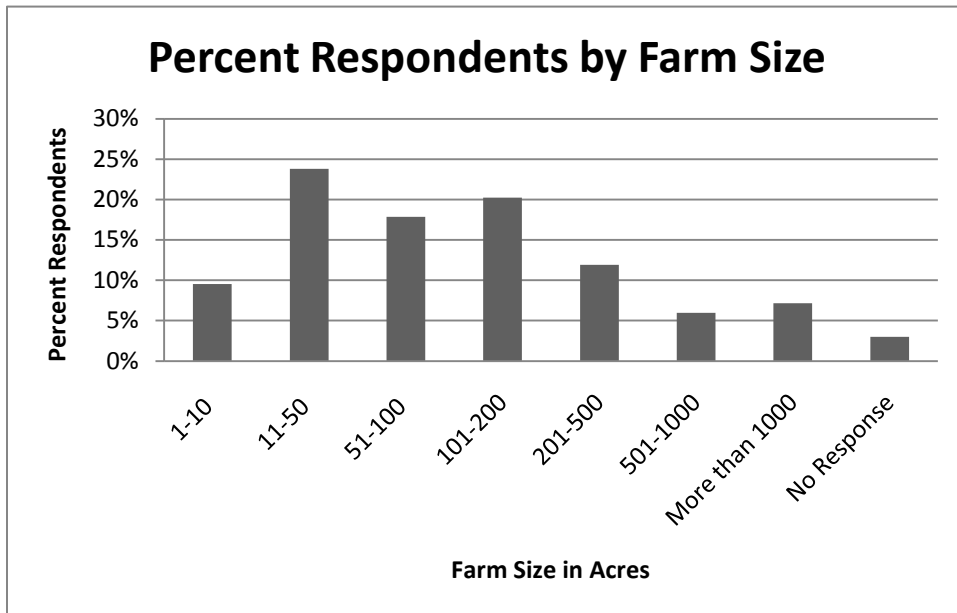


Figure 2-3. All farm sizes were represented in the survey with distribution that was not dominated by any one size. The average farm size in New York is 195 acres (USDA-NASS, 2007).

Surface Water Use, Method of Delivery, Management and Testing

Data collected revealed that 48 of 84 growers (57%) use surface water to irrigate their crops, while 15 of 84 (18%) report applying topical/pesticide sprays that are mixed with surface water. Of these growers who report using surface water to irrigate, 41 of 48 (85%) report that they apply the water overhead as one of their delivery methods or their only delivery method. Crops that are being irrigated with surface water that is applied overhead and/or have topical sprays that are mixed with surface water applied include all of the crops identified as high risk by the Food and Drug Administration (berries, green onions, herbs, leafy greens, netted melons, and tomatoes) as well as apples, beans, beets, broccoli, cauliflower, corn, cucumbers, eggplant, garlic, pears, peppers, potatoes, shallots, smooth melons, squash, and sweet corn. Of the growers who identified they were using surface water to irrigate and applying the water overhead, only 11 of 41 (27%) indicated that they were testing this water in any way, with 8 of 11 (72%) specifically indicating that they are testing for generic *E. coli*. The distribution of acreage being irrigated with surface water and the percentage of these operations using overhead as a delivery method is provided in Table 2-1.

Table 2-1. Overview of acreage irrigated with surface water and the percentage of farms using overhead irrigation as a delivery method.

Land Irrigated with Surface Water	# of Respondents	% of Respondents	% using overhead delivery
Less than 1 acre	2	2%	50%
1-10 acres	10	12%	50%
11-50 acres	15	18%	93%
51-100 acres	8	10%	100%
101-200	10	12%	100%
201-500 acres	1	1%	100%
501-1000 acres	2	2%	100%
None	36	43%	0%

Environmental Assessment

To determine how much growers were considering the impact of environmental conditions to water quality and produce safety, they were asked several questions regarding land and activities adjacent to their water sources and farms. When asked if they "have done an environmental impact of the area surrounding your irrigation water source to determine potential contamination factors?" noting that this included surveying the area around the water source to see if there was wild or domestic animal activity or man-made activity that could impact the microbial safety of the water source, 20% of the 84 respondents responded Yes, 55% responded No, and 25% did not respond. Twenty three percent of respondents (19 of 84) report adjacent land uses within one mile of their fields that may present a microbial risk including confined animal operations, landfills, dairy farms, horse farms or inadequate home leach fields/septic systems, while 17% (14 of 84) report using manure as a soil amendment within one year of harvest.

Discussion

Assessment of Water Risks

This survey highlighted several important factors regarding the use of surface water in the production of fresh produce and the impact food safety requirements might have on fresh produce growers. In New York, many fresh fruits and vegetables are overhead irrigated or have protective topical sprays mixed with surface water applied to them. Less than 20% of growers who indicated that they apply surface water overhead have tested the water they are using for generic *E. coli*, a commonly used indicator of fecal contamination in the determination of the

microbial quality of surface water. This is a concern for several reasons, beginning with need for growers to assess the risks their surface water may represent.

Testing surface water allows growers to define their current surface water quality and make informed decisions about when to apply or not apply irrigation and topical sprays that use the surface water. Water quality is one part of conducting a risk assessment that should include reviewing other management practices such as when water is applied in relationship to harvest and the method of delivery. Overhead application where the edible portion of the crop is contacted by the water and water applied within two weeks of harvest represent the highest risk practices if the quality of the water is poor. Without understanding water quality through testing, it would be difficult for growers to assess their risks. In addition, many topical sprays used in the production of fresh produce can and are applied with a 0 day to harvest (DTH) interval. Sprays such as Cuprofix Ultra 40 Disperss (copper sulfate), Switch (cyprodinil/fludioxonil), Elevate (fenhexamid) and Bravo Ultrex (tetrachloroisophthalonitrile) all have a 0 DTH interval and may be applied very close to harvest. Research has shown that pathogens present in poor-quality water can persist in pesticide mixes (Guan et al, 2001; Sathyanarayanan and Ortega, 2004). This importance of good water quality increases as the plants near harvest because there is less opportunity for UV solarization, desiccation, and other environmental factors to reduce microbial foodborne pathogens that may be present in the water. These risks can be reduced by applying irrigation water in the morning to promote exposure to the sun and drying of the crop (Steele and Odumeru, 2004). Again, making the best management decisions is based on having the right data regarding water quality. Water testing would allow growers to better monitor source water quality for changes or contamination events and allow them to make management decisions based on water quality information.

Environmental Assessment and Adjacent Land Use

Adjacent land and riparian zones can represent a risk to both the safety of water sources and fruits and vegetables grown in nearby fields. Survey results indicate that only 20% of respondents are doing an environmental assessment of the areas surrounding their water sources, even though 23% responded that their fields are within one mile of potential foodborne pathogen contamination sources that may present a microbial risk including confined animal operations, landfills, dairy farms, horse farms or inadequate home leach fields/septic systems, while other respondents indicated they had significant wildlife presence that may represent a risk or that they were applying manure within one year of harvest. A more worrisome result was that 51% of respondents to the question “Do you have any adjacent land uses within 1 mile of your fields that may present a microbial risk?” did not provide any response. It seems likely if the answer was none, they would have selected that answer so it seems more likely that they did not know, had not considered it, or did not want to reveal adjacent land issues, all of which are of concern. Foremost, it is important that fresh produce growers consider adjacent land use in their risk assessment. In some cases, identifying risks can be very difficult to manage because fields cannot simply be moved to a different location and other operations that represent risk cannot be asked to move or cease to exist. That said, the likelihood that something will be done to mitigate an adjacent land risk is much higher if the risk has been identified and is known, even though it may be difficult to fix. The survey results highlight a great opportunity to encourage growers to, at the very least, conduct an environmental assessment of adjacent land use as part of their farm food safety risk assessment.

Meeting Demands for Food Safety

Many New York and national retailers including Wegmans, Price Chopper, and Hannaford are all requiring that their “Locally Grown” providers have their food safety practices verified by a third party audit. These audits require that growers test their water sources, so the survey data indicates that many of the respondents are not participating in these third party audits. This notion is supported by the data collected that relates to the development and implementation of a farm food safety plan. Although almost 37% of growers (31 of 84) self-reported that buyers have inquired about food safety practices on their farms, only 17% of respondents said that they have a plan while another 10% reported that their plans are "in progress". Even if you combine these two groups, only 27% of participating farmers have a farm food safety plan or are working on a plan, while 37% are being asked about their practices. This indicates a large gap in what is being asked about or required, and in what growers are delivering. This could represent a market opportunity for those growers motivated to write a farm food safety plan, implement it, and successfully pass an audit. Eleven of 84 respondents (13%) report having had a third party audit to verify food safety practices on their farms. For those growers who continue to avoid the implementation of food safety practices such as testing their on-farm water sources, this could represent a loss of market or the need to find markets that do not have food safety requirements.

Economic farm viability could be impacted not only by losing markets but by litigation should a foodborne illness result as a consequence of consuming fresh produce grown without food safety practices in place. Although it is unlikely an individual farm may be indicated as the source of a produce related foodborne illness, farms that are implicated have been found liable and responsible for the financial impacts of illnesses (MarlerClark, 2010). Due diligence is defined as “the care that a reasonable person exercises to avoid harm to other people”. There is

no way to guarantee that fresh produce is 100% safe because it is grown outside under open skies in the soil, but growers do have a responsibility to understand risks that exist and implement practices to reduce these risks. Fresh produce growers should be aware of this liability and actively address it through the implementation of farm food safety practices.

Survey Assessment

One very important outcome of conducting this survey in both paper and electronic formats was noticing the stark contrast in the numbers of surveys returned through the postal service versus those submitted online. Only nine people completed the online survey, while 75 individuals completed the paper survey. As part of the survey mailing to 197 homes, we did include stamped return envelopes were included, but some of those who completed the paper survey paid for the postage to return the survey because they got the survey from a newsletter and not from the project directly indicating an additional investment of a stamp and an envelope over a free online submission. The exact return rate is impossible to determine because we are not certain how many surveys were distributed due to collaboration with extension educators who included information about the survey in their newsletters, but all news stories included the link to the electronic survey though they may not have included the paper survey. A review of the ages of those who submitted electronic versus paper surveys was conducted to determine if age may have been a factor. Of the nine electronic surveys submitted, 1 was from a 20-29 year old and 4 each from 40-49 and 50-59 year olds. Overall, the survey had participation across a range of ages (Figure 2-2), so there is no evidence that the preferential use of paper surveys was related to age. It is not clear why participants chose paper over electronic submission. However, it is very important to recognize the disparity since it could heavily influence participation in other surveys. This data highlights the value of offering paper versions of surveys to encourage participation by fruit and vegetable growers.

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Appendix 2-A

Dear Fresh Produce Grower,

Irrigation water management is of particular interest because of the changes that are occurring in the fresh produce industry related to irrigation water testing and management. As a result of the 2006 spinach-related outbreak of *E. coli* O157:H7, the leafy greens industry in California self-mandated irrigation water sampling and adopted strict standards for irrigation water quality. Most growers who are testing their irrigation water are doing so because they need the information to pass a third party audit of their food safety programs. Third party audits are requested by some buyers to verify their suppliers are following appropriate food safety practices. All of these issues are likely part of the reason that the Cabbage Research and Development Program (CRDP) committee ranked their two highest priorities in 2008 as (1) Food Safety and (2) Monitoring of Irrigation Water.

As part of a research project funded by the CRDP entitled *Monitoring Irrigation Water for Human Pathogens*, Cornell University researchers and extension professionals are conducting a survey of New York fresh vegetable producers to determine current irrigation water management practices related to food safety. Understanding current irrigation water management practices will assist us in developing water-sampling protocols that will fit with current management practices being used on the farm. Keeping up with industry water quality standards will help maintain the viability of New York farms. Survey results will also indicate how the New York produce industry may be affected if federal standards are mandated regarding irrigation water delivery and/or quality. Education and extension programs will be developed to proactively prevent any negative impact such regulations could have on the New York produce industry.

We appreciate your willingness to participate in this short survey. It is available in two formats; as an electronic document and as a paper document that can be sent through the US Postal Service. Please complete format you prefer. Here is the link to the electronic document.

<http://surveys.cit.cornell.edu/Survey.aspx?s=7e0f7b71ef914b87b08032363614953d&invitationID=27833>

As part of the Institutional Review Board created to protect you as a participant, we would like to remind you that participation in this survey is voluntary. We will not be collecting your name. You do not have to answer any questions that make you feel uncomfortable. Data generated from this survey may be used in research publications or extension presentations, but individual responses will not be presented such that the respondent is identifiable by name or demographic information. Raw data will not be released to anyone other than the project investigators listed in this letter. We have taken precautions to protect your submitted electronic survey, but all electronic documents are subject to unapproved viewing by third parties. This survey should take you approximately 15 minutes to complete. If you have any questions regarding IRB or your rights as a participant, please refer to this website <http://www.irb.cornell.edu/>.

We appreciate your willingness to participate. If you have any questions, please contact us.

Sincerely,

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Water Quality Management

Please take 15 minutes to complete the following survey and return it by **January 2, 2008**.

1. How many acres are you irrigating with surface water?

- a. None
- b. Less than 1 acre
- c. 1-10 acres
- d. 11-50 acres
- e. 51-100 acres
- f. 101- 200 acres
- g. 201-500 acres
- h. 501-1000 acres
- i. More than 1000 acres

2. What is (are) your source(s) of irrigation water? Please circle all that apply.

- a. Pond
- b. River
- c. Well
- d. Municipal
- e. Other, please specify: _____

3. What method of irrigation do you use? Please circle all that apply.

- a. Overhead
- b. Drip tape
- c. Micro jet
- d. Flood
- e. Furrow
- f. Other, please specify: _____

4. What crops are you irrigating with surface water? Please circle all that apply.

- a. Cabbage
- b. Lettuce
- c. Strawberries
- d. Raspberries
- e. Tomatoes
- f. Apples
- g. Grapes
- h. Green onions
- i. Herbs
- j. Netted Melons
- k. Smooth Melons
- l. Carrots
- m. Other, please specify: _____

5. Describe the frequency at which you irrigate your cabbage/crucifers in a “normal” year. Assume that frequency would increase in a dry year and decrease in a wet year.

- a. Never
- b. 1-2 times
- c. 3-5 times
- d. More than 5 times
- e. Weekly
- f. Other, please specify: _____

6. Do you apply topical/pesticide sprays that are mixed with surface water? Yes No

7. If you use surface water to mix topical/pesticide sprays, what is the source of your surface water? Please circle all that apply.

- a. Pond
- b. River
- c. Well
- d. Municipal
- e. Other, please specify: _____

8. Are you currently testing your surface water source? Yes No

9. If you are testing surface water, what are you testing for? Please circle all that apply.

- a. Generic E. coli
- b. Fecal coliforms
- c. Pathogens (If so, please specify the organism(s) _____)
- d. Nitrates
- e. Other, please specify: _____

10. How often do you test your surface water water?

***Note:** If you test different sources of water on different schedules please list each source individually as well as how often you test the water. For example: pond water – once per growing season.

11. Have you done an environmental impact of the area surrounding your irrigation water source to determine potential contamination factors? Yes No

***Note:** This includes surveying the area around the water source to see if there is wild or domestic animal activity or man-made activity that could impact the microbial safety of the water.

12. Do you have a written farm produce safety plan?

Yes No In progress

13. Have any buyers of your commodities/crops ever inquired about food safety practices on your farm? Yes No

14. Have you ever had a third party audit of your farm to verify food safety practices?

Yes No

15. Do you have any adjacent land uses within 1 mile of your fields that may present a microbial risk? Please circle all that apply

- a. Confined animal operations
- b. Land fills
- c. Dairy farm
- d. Use of manure as a soil amendment within 1 year of harvest
- e. Other, please specify: _____

Demographic Information

Age

- a. under 20
- b. 20-29
- c. 30-39
- d. 40-49
- e. 50-59
- f. 60-69
- g. over 70

County _____

Size of farm

- a. less than 1 acre
- b. 1- 10 acres
- c. 11-50 acres
- d. 51-100 acres
- e. 101-200 acres
- f. 201-500 acres
- g. 501-1000 acres
- h. More than 1,000 acres

Please share with us any comments or concerns regarding the safety of irrigation and spray water used on vegetable crops?

Thank you for your time!

If you have any questions about the survey, please contact Betsy Bihn at eab38@cornell.edu or (315) 787-2625.

Chapter 3

Development of an Irrigation Water Quality Database to Identify Water Resources and Assess Microbiological Risks during the Production of Fresh Fruits and Vegetables

Introduction

Fresh and minimally processed, ready-to-eat fruit and vegetable production is a multi-billion dollar industry in the United States (Kaufman et al, 2000). These commodities are often irrigated with surface water throughout the US (Suslow et al, 2003). While there is concern with all sources of water for pre-harvest use, relative to food safety, surface water is more likely to be exposed to human and animal fecal contamination than ground water and is expected to pose greater risk to human health than irrigation with water from deep aquifers with properly constructed and protected wells (Brackett, 1999; Steele and Odumeru, 2004). Surface water used for the production of fresh fruits and vegetables has been found to be contaminated by many human foodborne pathogens such as *Salmonella*, *E. coli* O157:H7, *Giardia*, and *Cryptosporidium* (Steele and Odumeru, 2004; Chaidez et al, 2005; Duffy et al, 2005; Izumi et al, 2008).

Previous studies of irrigation waters have been concerned primarily with chemical rather than microbiological water-quality parameters (Seiler and Skorupa, 2001). As a result, there is a nationwide knowledge gap regarding sanitary quality of irrigation waters. Public attention to recent outbreaks of foodborne illness has led some commodity groups to self-mandate irrigation water sampling and set quality standards based on the United States Environmental Protection Agency's Ambient Water Quality Standards (USEPAAWQS) for fresh water (USEPAAWQS, 1986). Recreational-water criteria was developed for water used for recreation that results in full body contact by people and it accepts that some individuals will get sick with gastrointestinal

illness. The levels in the USEPAAWQS estimate that 8 illnesses per 1,000 swimmers will result at fresh water beaches even when the standards are met.

The USEPAAWQS may not be appropriate for direct application to irrigation water but in the absence water data related to fresh produce production, this standard was adopted. The Commodity Specific Food Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens (CSFSGPHLLG) is one industry guideline that has modified the recreational water standards for use in fresh produce production. In addition to industry adoption of these standards, in December 2009 the United States Food and Drug Administration publicly announced their intention to develop a Produce Safety Regulation for fresh produce and the Food Safety Modernization Act, signed into law on January 4, 2011, also has provisions for produce safety regulations. The draft of this FDA regulation is scheduled for release in December 2011 and may contain testing parameters or water quality standards for surface water quality used during the production of fresh produce.

Developing an Irrigation Water Quality Database will begin to fill the knowledge gaps about water quality that exists. Preliminary research data gathered prior to the beginning of this project from surface water sources used to overhead irrigate fresh produce crops indicated that if growers were forced to adopt the USEPAAWQS, they would either have to discontinue the use of some of their water sources or implement mitigation strategies to reduce the microbiological load because surface water quality can vary over the season (Bihn, unpublished data). These mitigation strategies could represent a significant financial investment and directly impact farm viability. Both food safety and the importance of water as a natural resource are being managed on the farm and understanding current water quality will allow farmers to make informed decisions about surface water use.

This project aims to provide an objective assessment of the sanitary quality of surface water currently used for irrigation in New York State through the collection of water quality parameters including quantified generic *E. coli*, specific conductance, pH, and turbidity. Investigating water quality over two years, through three seasons each year, provided useful insights but also highlighted areas where improved sampling strategies could be adopted to further our understanding of surface water quality. Harvest seasons are known to impact the presence and abundance of pathogens in irrigation water, though these changes are not always found to be reflected in the microbial load on the fresh produce that is grown at the same time (Selma et al, 2007). Understanding seasonal and source fluctuations provides information that can improve on-farm irrigation water management practices by fruit and vegetable producers. Overall, the database will improve our understanding of current water quality, allowing for better on-farm risk assessment, while providing strategies for implementing an effective water testing program. Resulting educational materials including the water sampling protocol and extension trainings improve grower understanding of water testing expectations, the ability to interpret water testing results and provide assistance for understanding when mitigation strategies should be adopted.

In addition, this database was developed to facilitate participation from others interested in providing water quality data so that a nationwide estimation could be developed. With participation from collaborators in Tennessee and Texas, the scope of the database has been expanded and additional participation from other collaborators remains a prospect. Comparing data when possible and cataloging water testing results, continues to build a much needed assessment of surface water quality.

Materials and Methods

Irrigation Water Database Development

The irrigation water quality database is a FileMaker 10 data collection system. The database is comprised of 6 independent, relational files hosted on a FileMaker server. It was designed to facilitate data gathering from multiple sources such as independent laboratories or researchers at other Land-Grant Universities. Two of the files (data entry and grower address) are designed to be web accessible allowing an individual with proper permissions to access the data entry file via a web browser (e.g. Internet Explorer, Firefox, Safari, etc.) without the need to have the FileMaker program installed on their personal computer. The remaining files have administrator privileges and are not viewable to those entering the data.

Each grower who participates in the program is assigned a 9-digit, numeric grower code in an effort to ensure the participant's confidentiality. The “Grower” file stores the grower's contact information, commodities grown, and water source(s). The state and county information for each grower, is also recorded. Much of this information was used to generate a water sample collection form which included the unique 9-digit, grower ID that appears on each form.

The collection forms were given to those collecting water samples and in turn were submitted to the laboratory for analysis. The completed laboratory forms were then given to the data entry operator for entering into the FileMaker system. Upon registering with the program to provide data, individuals were provided with a database tutorial to instruct them on how to access the data entry portal (Appendix 3-A). They were provided with an individualized password and login to gain entry to the database.

To enter data, the data entry operator was required to login into the database and enter the grower ID that appeared on each form. The operator would enter all of the laboratory data from

the water collection form and submit it for each grower. Multiple forms were required if an individual grower had more than one water sample source or sampling location. Each sample was entered separately. After all collection forms had been entered, the data entry operator would log out of the system.

When the database administrator logged into the water collection file, all grower collection data was imported automatically from the data entry file into the collection file. Once the import process was complete, all records in the data entry file were erased, thereby preventing the possibility of further access to the entered data. Only those with access to the water sample collection file could view the collected data. The data imported into the collection file was “as entered” by the data entry operator. If there was a mistake, the data entry operator was required to contact the database administrator to correct the data. If an administrator needed to correct entered data, there was a standard protocol in place for making corrections that included entering the user’s ID, date, and reason for the correction. This information became part of the permanent record so that it was obvious that the data had been modified. The irrigation water quality database was developed so that it could evolve as needs of users evolved.

Water Collection

A standardized water sampling protocol was developed, tested, and used as a training protocol (Appendix 3-B). Four Department of Agriculture and Markets personnel and a summer intern were trained with the standardized water sampling protocol to facilitate sample collection across the state. One of the trainings utilized remote video conferencing.

One liter of water was collected from each site into either bottles cleaned or purchased following a protocol that resulted in decontamination of the bottles (EPA protocol “B”). Collected samples were placed in a cooler with ice packs and either delivered directly or sent via

overnight delivery to a laboratory for analysis (NYS Food Laboratory or Cornell laboratory). The overnight delivery caused sample analysis to fall outside of an 8 hour analysis window, but according to Pope et al (2003) samples held at 10°C and not frozen for up to 48 hours generated comparable results (Pope et al, 2003).

Sample Analysis

Samples were analyzed for quantified generic *E. coli*, specific conductance, turbidity, and pH. Other data points related to water collection included the date of sample collection, type of water source, the name of the water source if it is a named body of water, such as a stream or lake, and the code number for the grower. These data collection points were evaluated in the first year while the database was populated and tested.

Generic E. coli Quantification

Idexx Quanti-Tray sealer (Westbrook, ME) was turned on and allowed to warm so that the machine was ready to receive samples. Idexx Quanti-Tray 2000 cards and vials were labeled with identifier on sample bottle and date of test. Each water sample was inverted completely 25 times and 100 mLs of sample were aseptically transferred into Idexx Colilert vial (Westbrook, ME). Colilert substrate was added into each sample vial. Samples were aseptically capped and mixed by inverting 25 times then allowed to sit for approximately 5 minutes to allow the substrate to dissolve. Each sample was poured into Quanti-Tray card with the matching label, taking care not to touch vessel to card. The card was tapped gently on bench to release bubbles then the Quanti-Tray card was seated in the holder and water was forced into upper wells by applying hand pressure in a sliding motion towards the top of the card. The Quanti-Tray card was sealed by passing it through the sealer. It was incubated at 35°C for 24 hours, verified by

recording the start time on the card. Yellow colonies and fluorescing wells at 366 nm were counted using Comparator card and counts were recorded. Final MPN were determined by referring to IDEXX Quanti-Tray/2000 MPN table.

The Colilert system was selected for this project because it was easy to use and enumerate, and has a low false positive rate (Brooks et al, 1998). Not all verotoxin-producing *E. coli* will test positive in this system, but the conclusions that were reached in this project are not based on sampling for pathogenic *E. coli*. Current Good Agricultural Practices recommendations for fresh fruit and vegetable growers suggest testing for quantified generic *E. coli* and this system is acceptable for this purpose, as well as for the overall funding available and collaboration required for this project.

Specific Conductance

Specific conductance measures water's ability to conduct electricity, normalized to a temperature of 25°C to reduce the confounding effect of variable temperatures among bodies of water or different seasons. It results in a measurement expressed as microSiemens per centimeter ($\mu\text{S}\cdot\text{cm}^{-1}$) and is generally found to be a good measure of the concentration of total dissolved solids (TDS) and salinity. It was included in this project as an indicator of run-off events that could impact water quality as run-off events typically would cause a decrease in the specific conductance of a particular water source.

Samples were allowed to sit for approximately 24 hours in the dark at ambient temperature. The sample to be tested was inverted 25 times prior to pouring. An empty 100 mL sterile dilution blank bottle was initially rinsed with a small amount of the sample to be tested and then filled $\frac{1}{2}$ to $\frac{3}{4}$ volume of bottle with the sample. Conductivity meter was calibrated with a traceable conductivity standard. The conductivity meter's electrode was immersed into the

water sample and the result was recorded once the reading stopped fluctuating and remained constant. Between samples, the electrode was rinsed with distilled water and dried with tissue paper. The meter read zero prior to additional samples being tested.

Turbidity

Sample bottles were allowed to sit overnight in the dark at ambient temperature. Each sample was gently inverted 25 times and the sample cell was filled to the analysis line. Samples were degassed using a rubber stopper and syringe. The exterior of the sample cell was cleaned with cheesecloth, a drop of silicone oil was applied and spread uniformly with a soft lint-free cloth. Sample cells were held at the top to avoid leaving fingerprints on the cell. Analysis was completed on a Hach 2100P portable turbidimeter (Loveland, CO) set to “auto rng” mode. Samples were inserted with its orientation marking (*) facing forward and the lid was closed. When the “Read” button was pushed, the Nephelometric Turbidity Units (NTU) reading appeared and was recorded.

pH

A BeckmanΦ720 pH meter (Brea, CA) was calibrated daily. Each sample was gently mixed by inverting the bottle, then the electrode was placed in the sample and the pH measurement was taken. All results were recorded on the sample submission form.

Results and Discussion

Data Analysis

In the New York data set there were a total of 270 samples collected from 15 counties (Figure 3-1). These samples were taken from wells, rivers, streams, canals, swamps, lakes, and

ponds. To aid in analysis, sometimes this larger data set was broken into three distinct categories; ground water representing wells; running water representing rivers, streams, and canals; and reservoir water representing lakes, ponds, and swamps. Wells usually represent ground water that should be protected since it is not open to the environment. Though not all wells may be properly capped or protected, for the sake of analysis in this chapter, they are viewed as a specific data set. Some analysis was done by year or by season to determine if trends were present or variation existed.

The database contains New York data as well as data from Tennessee (62 samples) and Texas (92 samples). Tennessee data was collected only in 2010 but used similar collection parameters and were analyzed for comparison when appropriate. Texas data was not analyzed since the collection parameters were significantly different and did not warrant reasonable comparisons.

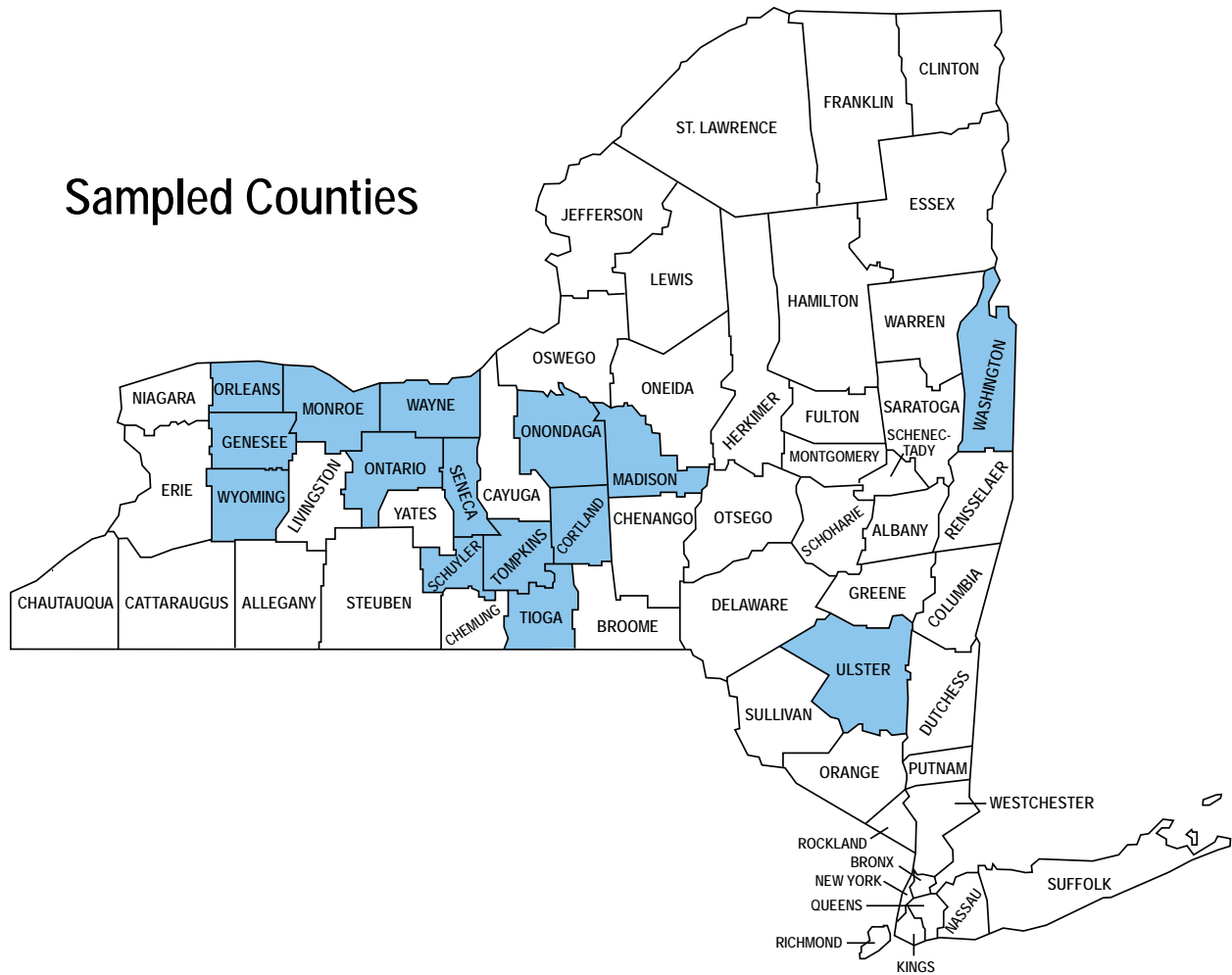


Figure 3-1. Water samples were collected from 15 counties throughout New York State. Counties with participating farms are highlighted in blue and data generated from the sampling was used to initially populate and test the Irrigation Water Quality Database.

General Results

Two hundred seventy surface water samples were collected and analyzed for pH, specific conductance, turbidity, and quantified generic *E. coli* using the Colilert Quantitray 2000 method resulting in most probable number (MPN) per 100 mls. A cohort of 254 samples were divided into ground water (23 samples), running (94 samples), and reservoir groups (137 samples). Of the remaining samples, one sample was not properly analyzed for generic *E. coli* due to an incubator malfunction and 15 were sampled directly from irrigation equipment and their removal

from the predominant data set will be discussed in detail later in the chapter. Reporting the geometric mean was the standard currently being used by both the USEPAAWQS and the CSFSGPHLLG, so it is the standard used for reporting in this chapter (Dufour and Schaub, 2007; CSFSGPHLLG, 2010). Medians and averages were also calculated (data not included). Median calculations aligned very closely to the geometric mean in most cases while averages were usually much higher since calculating the average does not manage extreme data points in the data set like the geometric mean calculation.

Overall, ground water had a geometric mean of 1 MPN/100 mls, reservoir water had a geometric mean of 8 MPN/100 mls and running water had a geometric mean of 52 MPN/100 mls. One hundred percent of the time, the ground water samples were below the 126 MPN/100 mls USEPAAWQS standard that is also used by the CSFSGPHLLG. Reservoir water met the 126 MPN/100 mls standard 96% of the time with only 3% of the samples exceeding the 235 MPN/100 mls standard which is the single sample upper limit for water intended for foliar applications to the edible portion of the crop as set forth in the CSFSGPHLLG water standards (CSFSGPHLLG, 2010). Running water samples met the 126 MPN/100 mls standard 74% of time with only 15% of the samples being higher than 235 MPN/100 mls standard. Though 3% for reservoir water and 15% for running water seem rather low, it does indicate that there were samples that exceeded the limit and would therefore require mitigation including retesting or abandoning the water source. Both of these have serious water management and financial implications.

Further analysis of the water quality data from New York (NY) by year reveals consistent results from year to year and source to source (Figure 3-2). Ground water had the lowest counts with running water having the highest counts. Reservoir water samples had almost an order of

magnitude less *E. coli* than running water sources even though reservoir water sources are open to the environment like running water sources. Unlike reservoir sources, running water sources are subject to a multitude of variables due to the nature of running water sources having miles of banks with uncontrolled access by wildlife, septic systems, drainage tiles, run-off, and/or human recreational activity.

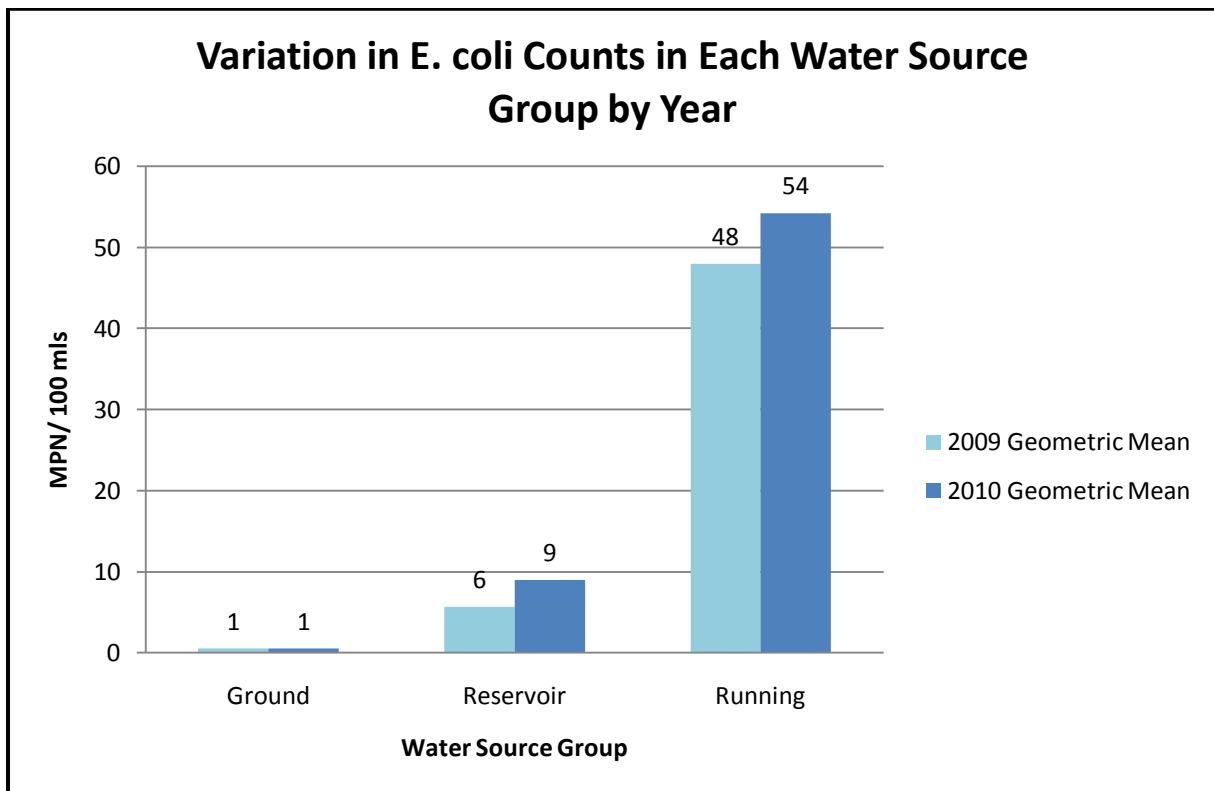


Figure 3-2. Geometric means of each New York State water source group by year.

Comparison by State and Season

Collaborators in Tennessee (TN) began participating in the Irrigation Water Database in 2010. There were 66 samples collected in TN and entered into the database with 20 ground water samples, 27 reservoir samples, 16 running water samples and three municipal samples. The municipal samples were not included in the statistical analysis because there was no data to

compare it to in New York, but interestingly one of the samples contained generic *E. coli* (2 MPN/100 mls). Several samples from TN were taken from irrigation equipment. In the New York data set these samples were removed because in many cases there were samples taken from the source water at the same time. Since there were fewer TN samples, the samples taken from irrigation water equipment were included in the analysis. TN water samples were analyzed for the same parameters as the NY samples but modified mTec (EPA 1603) protocol was used for quantification of generic *E. coli* resulting in colony-forming units (CFU) per 100 mls. Despite the different protocols, the results of NY and TN were compared on a one to one basis. This is supported in the scientific literature as noted by Cho et al, 2010 when they reported a positive relationship between CFU and MPN estimates.

Overall, TN ground water had a geometric mean of 1 CFU/100 mls, while TN reservoir water had a geometric mean of 5 CFU/100 mls and running water had a geometric mean of 38 CFU/100 mls. One hundred percent of the time, the ground water samples were below the 126 MPN/100 mls when allowing a one to one comparison. Reservoir water met the 126 MPN/100 mls standard 93% of the time with only 4% of the samples exceeding the 235 MPN/100 mls single upper limit standard. These percentages are consistent with those seen in NY water sources. TN Running water samples met the 126 MPN/100 mls standard 75% of time with only 6% of the samples being higher than 235 MPN/100 mls standard. The percentage of samples achieving the 126 MPN/100 mls standard is comparable to NY while the percentage of samples that exceed the 235 MPN/100 mls limit is approximately half of what was seen in NY. A possible explanation for this variation is that TN running water samples accounted for the smallest analyzed sample size at 16 samples. This issue of small sample size also caused an anomalous result when attempting to access seasonal variation.

Analysis of TN running water data in the fall (September 22-December 20) resulted in an excessively high geometric mean of 255 CFU/100 mls. This is the result of calculating the geometric mean with only three samples. In general, a five sample minimum is preferred for calculating a geometric mean. The three samples were 860, 160, and 120 CFU/100 mls, all taken from the same water source on the same day, what is interesting to note is the variation and the fact that one of the samples would meet the 126 MPN/100 mls standard and the 160 CFU/100 mls sample does not exceed the 235 MPN/100 mls limit. These results likely would not have caused the grower who was testing the water to do anything, while the 860 CFU/100 mls result exceeds even the most liberal EPA recreational water standards of 575 MPN/100 mls for “Infrequently Used Full Body Contact” water and it also exceeds the upper limit in the CSFSGPHLLG standards (576 MPN/100 mls) for water that does not contact the edible parts of the plant such as water delivered through a drip system (USEPAAWQS, 1986; CSFSGPHLLG, 2010). With a clear understanding of these issues, this data was included in the comparison of seasons and states found in Figure 3-3. Unlike NY, TN running water samples are not always higher than the TN reservoir samples. In NY, *E. coli* counts in both running and reservoir water are highest in the summer (June 21-September 21), while spring (March 20-June 20) resulted in the highest counts in both reservoir and running if you discount the fall running data due to the issues discussed earlier in this section. To be confident in the TN data, more samples are needed to make the evaluation more meaningful. The NY data shows seasonal trends in both running and reservoir water with the changes more pronounced in the running water from season to season. Encouraging participation in the database from more states would allow for additional data analysis and may reveal trends that are regionally and/or seasonally dependent.

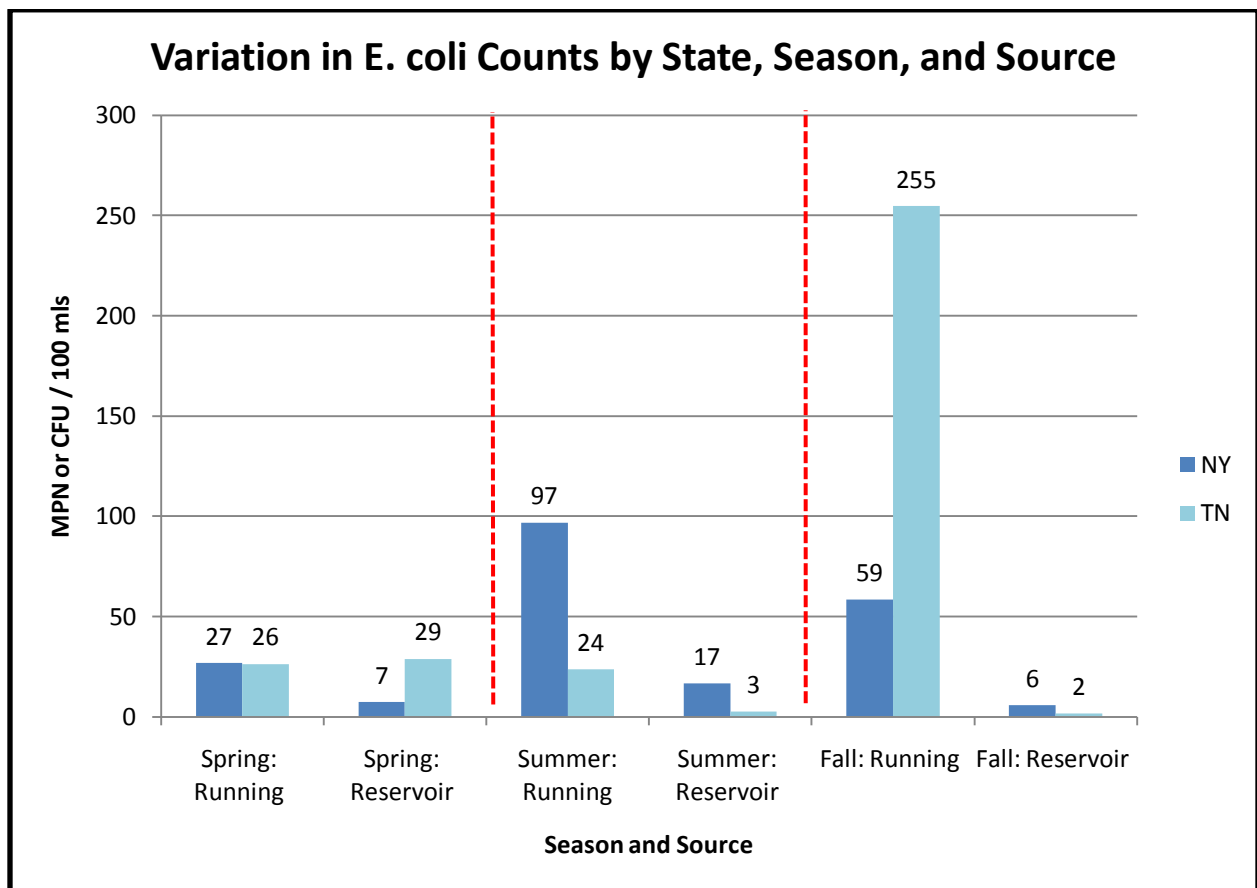


Figure 3-3. New York and Tennessee data was analyzed by season. Spring is represented by samples taken between March 20-June 20, summer is represented by samples taken between June 21-September 21, and fall is represented by samples taken between September 22-December 20. No samples were taken in winter (December 21-March 19) and NY samples from 2009 and 2010 were combined for this analysis.

Impact of Sampling Location

For all water collections, the sample location was noted. Most samples were taken directly from the source water, but there were several opportunities in the 2010 NY sampling season to collect water samples directly from irrigation equipment. The idea behind sampling from irrigation equipment was to collect water closest to the point of use and get the best possible data on the quality of water that was contacting the plant. Data generated in this study found that this may not be the best way to determine source water quality but may be relevant to

understanding overall risk. During the 2010 season there were nine samples collected from irrigation equipment and the source water that supplied the irrigation equipment. Three out of the nine samples resulted in the irrigation equipment sample having higher *E. coli* counts than the source water sample beyond the 95% confidence limits (Table 3-1). The other six samples fell within the 95% confidence limits, though two of the samples were closer to the limits than the other four.

Table 3-1.

Sample Location	MPN/100 mLs	95% Confidence Lower limit	95% Confidence Upper Limit
Source water	13.5	7.8	21.8
Drip irrigation	25.6	17.2	35.7
Source Water	110.6	81	148.8
Drip Irrigation	90.9	66.6	121.6
Source Water	1.0	0	3.7
Drip Irrigation	6.3	2.5	12.7
Source Water	727.0	475.7	1048.9
Overhead Irrigation	1203.3	810.8	1750.7
Source Water	125.9	102.1	152.3
Drip Irrigation	298.7	207.1	423.2
Source Water	74.3	53	98.8
Drip Irrigation	72.3	51.5	96.4
Source Water	<1	0	3.7
Drip Irrigation	>2419.6	1439.5	infinite
Source Water	42.6	28.7	60.7
Overhead Irrigation	49.6	35.4	67.8
Source Water	16.1	9.6	24.9
Overhead Irrigation	16	9.5	25.1

Although there were only three samples from irrigation equipment that differed from their source water samples, it highlights two important issues. The first issue is that irrigation equipment could add risks to the production of fresh produce through the addition of microbial contamination that does not exist in the source water. Two of the samples were from farms using

drip irrigation and the increase in counts could be related to soil conditions including the use of amendments that increase bacterial counts since the drip tape is in direct contact with the soil. The overall risk to the food production system is low because the water is applied at or just below the soil line and primarily wets the soil, not the edible portion of the plant. One of the irrigation equipment samples with high *E. coli* counts when compared to the source water was collected from overhead irrigation equipment. In the database, the grower identified that this water was used to irrigate leafy greens. The *E. coli* counts in both the source water and the irrigation equipment significantly exceeded the EPA recreational water standards and since this water was identified as being applied overhead, it represented the riskiest application method due to wetting of the edible portion of the crop. It is not known whether the grower was actually irrigating with this water and how close to harvest it was applied, but it clearly represents concerns that the grower should review. In the risk assessment, the growers using both systems should review the siphon system that feeds the irrigation pipe to be certain it is suspended in the water and not siphoning bottom sediment as this is known to increase the microbial content (Badgley et al, 2011).

The second important issue is to understand that sampling from the irrigation equipment may not accurately reflect the quality of the source water and as mentioned above, should cause a grower to review other aspects of production such as water delivery systems. This is important because financial and time resources are limited and growers should be targeting resources to mitigating the risks. Modifying a siphon float, flushing the irrigation lines, or reviewing soil amendment application time lines instead of treating the entire water source may be easier, less expensive, and reduce risks more effectively, but without testing both the source water and the irrigation equipment, the grower would not know where the greatest risk exists.

In the CSFSGPHLLG (2010) it instructs farm operators to "sample sources as close to the point-of-use as practical, as determined by the sampler to ensure the integrity of the sample". Based on the inconsistent data generated when sampling irrigation equipment, it may be that the sampler could determine that in order to ensure the integrity of the sample, it should be sampled from source water, not the equipment. An alternative solution would be to collect two samples, one from the source water and one from the irrigation equipment, but even this has its challenges. Although irrigation systems may feed off of the same main pump line, most systems branch and thus have multiple end points and that type of testing strategy would increase the number of samples and the cost substantially.

These complicated scenarios that do not have scientifically supported testing strategies or assessment of actual risks, rightfully frustrate growers who want to do the right thing but are hesitant to invest time and money in testing that may not be relevant. Results from this project provide important data points that add key information to the discussion. If funds and time to test water are limited, testing source water would be the best use of resources, particularly if the method of irrigation utilized is drip or furrow.

Correlations

Analysis was conducted to determine if there were strong or weak correlations between the *E. coli* and the other parameters that were collected including specific conductance, turbidity, and pH (Table 3-2). These correlations were only determined for the surface water sources sampled at the source water. There was a very weak negative correlation observed between *E. coli* levels and pH, in all water surface water samples over the two years ($r = -0.18$) and a very weak positive correlation between *E. coli* and turbidity ($r = 0.05$) and specific conductance ($r = 0.12$). When running water and reservoir water were analyzed separately, some of the

correlations were stronger. The strongest correlations identified were between *E. coli* and specific conductance ($r=0.20$) and *E. coli* and pH ($r=-0.28$) in reservoir water, and *E. coli* and turbidity in running water ($r=0.21$), but these correlations are not sufficiently significant to be dependable indicators of the presence or quantity of *E. coli*.

Table 3-2.

NY Surface Water Sources	<i>E. coli</i> / specific conductance	<i>E. coli</i> /turbidity	<i>E. coli</i> /pH
Reservoir	0.20	0.05	-0.28
Running	0.00	0.21	-0.05
Reservoir and Running Combined	0.13	0.05	-0.18

Informed Growers

One of the outcomes of this project was the development of a protocol for sampling water sources used in the production of fresh fruits and vegetables. As discussed in the methods section, a modified version of this protocol was used to train individuals who participated as sample collectors in this project, but it was predominantly used during extension trainings to train growers how to collect samples for submission to water testing laboratories. Fresh produce growers who participated in this project benefited by having baseline water sampling completed for their on-farm water sources. They were supplied with a water testing protocol and had direct access to extension personnel to discuss water quality issues on their farms. Other NY farmers benefited through the trainings that were conducted to help them understand the value of water testing, how to test their water sources, and how to locate laboratories that can provide the analysis that they need. Some results from this project suggest that growers should consider expanding the water testing analysis that they request from laboratories.

Ponds are a very common source of surface water in New York and in this study the average *E. coli* counts in ponds was a geometric mean of 6 MPN/100 mls. In one particular case, a farm pond had an unusually high *E. coli* water test (>2419 MPN/100 mls) so contact was made with the grower to discuss what could have been the cause. One of the farm owners participates in the Community Collaboration Rain, Hail, and Snow Network (CoCoRaHS), so they were able to share that the night before the sample was taken, they had 0.2 inch of rain. A review of the data showed all the indications of a rain event in that the turbidity was higher and the pH and specific conductance were lower indicating dilution by rain. The other factor for this sample was that it was taken directly from the irrigation equipment which in other instances in this study has also caused an unusually high *E. coli* count. A follow-up sample had much lower *E. coli* result (3.1 MPN/100 mls) and was not a concern to the grower. This farm's participation in CoCoRaHS gave them a monitoring tool they did not know they had. In addition, being able to review the turbidity, specific conductance, and pH data provided a level of confidence in the determination of a rain event. Understanding the impact rain has on water quality attributes and the possible influence of irrigation equipment on water tests can be important for making management decisions about water use. Since this farm uses drip irrigation, the risks from contaminated water are substantially lower than if the farm used overhead irrigation that resulted in the edible portion of their crops being contacted by water.

Another important monitoring result was collecting both *E. coli* and coliform data. Coliforms are usually not a good indicator for farm environments because many natural non-pathogenic plant, soil, and water microorganisms are included in the coliform count (Brackett and Splittstoesser, 2001; USEPA, 2010), but having a coliform number with an *E. coli* number proved valuable in figuring out contamination on one particular farm in this study. The on-farm

pond was filled naturally but also filled from a well to keep it as a viable irrigation water source. Many farms will fill ponds with well water to create a water store since many wells cannot provide the volume on demand needed to run overhead irrigation equipment. In the four previous water samples, this pond had a geometric mean of 7 MPN/100 mls (average 16 MPN/100 mls) but this water sample was 1299.7 MPN/100 mls. A review of the data showed that the coliforms on this date were 1011.2 MPN/100 mls, almost a one to one ratio of *E. coli* to coliforms indicating direct fecal contamination of the pond. In fact, the coliform test should have been higher as *E. coli* should be enumerated in the coliform test as well. At the follow-up sampling, it was noted that a well was being drilled on the property approximately 300 feet from the pond, but consultation with the grower highlighted the more likely issue. Once the information about the ratio of *E. coli* to coliforms was shared and explained, the grower identified an underground manure slurry piping system that emerges 15 feet from the pond. This piping system is used to carry manure slurry from a collection location throughout the farm and the field lines can be toggled from the manure line to the irrigation line from the pond. Upon switching between the lines there can be a release of manure slurry and with the short 15 foot distance, it seems likely this could have been the source of the contamination. A risk-assessment of the water use revealed that the grower had only been using this water to irrigate turf which is not a source of human food. This information may lead the grower to implement different management strategies for switching lines in the field in an attempt to reduce the risks to the open pond. The follow-up water test resulted in a <1 MPN/100 mls for *E. coli* and >2419.6 for coliforms.

The purpose of sharing these examples is to highlight that growers need to be aware of things that can impact water quality and how to interpret water testing data. This project

collected parameters outside of the current recommendations of a generic *E. coli* test, but adding them would not significantly increase the water test costs but may provide growers with additional information that can assist them with determining the source and cause of contamination.

Recommendations for Growers

In drawing conclusions from this research it is important to consider if any of the results warrant the modification of current recommendations. Based on weak correlation data, it is clear from the research that the only way to know the quality of a surface water source using *E. coli* as the indicator organism is to test the water source for *E. coli*. The other parameters evaluated including pH, specific conductance, and turbidity are not, in themselves, sufficiently adequate indicators of water quality or of presence or concentration of *E. coli*. Though there is much discussion about the value of generic *E. coli* as an indicator organism, it is the best available at the moment and there are laboratories that can complete the testing for growers (Suslow, 2010). If a new indicator organism is developed it will likely take significant time for laboratories to offer the test and may result in increased testing costs, so it is important to consider that growers are not researchers and must have access to affordable and meaningful testing strategies.

One modification to the current recommendation of testing surface waters that seems indicated by this research would be to add additional parameters beyond quantified generic *E. coli* that do not substantially increase the cost of the test but would provide the growers with additional information that might allow them to understand their test results. Specifically, specific conductance (\$10) and turbidity (\$8) would allow them to understand if run-off is influencing their test results. Although, the value of pH for water quality issues was not viewed as significant enough to recommend, some growers who signed up to participate in this study did

so in order to determine the pH of their water sources because they were interested in the information due to how it impacts their spray mixes. Since it is a relatively inexpensive test (\$5) that can provide useful information to growers, including it in the recommended tests seems reasonable. The costs quoted for tests were provided by Certified Environmental Services Inc., a commercial water testing laboratory in Syracuse, NY.

The amount of testing and the timing of the testing is another area that warrants discussion. The 1986 EPA Ambient Water Quality Standard Criteria (EPAAWQSC) states the sampling frequency and testing to determine the quality of the water should be “based on a statistically sufficient number of samples (generally not less than 5 samples equally spaced over a 30-day period)”. This was adapted for use in the Commodity Specific Food Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens (CSFSGPHLLG) as part of the California Leafy Greens Marketing Agreement (LGMA). As it appears in the decision tree for pre-harvest water used for foliar applications that result in the edible portion of the crop being contacted by water (i.e. overhead irrigation, topical protective sprays, frost protection), it states “Sampling Frequency: One sample per water source shall be collected and tested prior to use if >60 days since last test of the water source. Additional samples shall be collected at intervals of no less than 18 hr and at least monthly during use. Geometric means, including rolling geometric means shall be calculated using the five most recent samples”.

The sampling strategy set forward by the LGMA is more reasonable for agriculture than five samples spaced over 30 days as in the USEPAAWQS, but the calculation of the rolling geometric mean of 5 samples increases the testing requirements. Some growing seasons are particularly short in NY, so in order to get five water samples over the season, growers would need to sample at least once per week and even at that frequency, they would not be able to

calculate their first geometric mean until the season was almost over. The single sample standards are more relevant in short production seasons, but because they are based on the recreational water standards they accept that some people will get ill. Although there are no risk-free fresh fruits and vegetables, many in the media and government are prone to promulgating the idea that no one should ever get sick while eating.

Understanding and accepting that there are risks that come from using surface water sources during production is key in assessing the value of surface water sources. Using water quality standards such as the EPA recreational water standards that clearly accept some illness as an outcome may seem unwise but requires additional consideration. Unlike full body contact water that assumes individuals will be directly exposed to the water and ingest some of it, surface water used for irrigation may never be ingested by those who consume the fresh fruits and vegetables. Irrigation water is often applied days before harvest so the water is dried by the time the fresh produce is picked. Exposure to the sun and desiccation promote the reduction of microbial populations that may be deposited by the irrigation water, so the risk is less than recreational waters (Steele and Odumeru, 2004).

Another consideration that supports the use of surface water for fresh produce production is the world-wide shortage of clean drinking water. If growers are driven to use water that has zero microbial load, they will either have to treat the water or move to ground or municipal water. The use of ground or municipal water in the US may not seem like such a dire option at the moment, but already in states like Florida and California, municipalities have established reclaimed water distribution systems to encourage people to not waste drinking water on watering lawns, gardens, and crops. When countries outside the US are considered, the notion of using a clean drinking water source to irrigate crops is in some cases unthinkable. The World

Health Organization sets the standard for wastewater used to irrigate crops that will be eaten raw at < 1,000 fecal coliforms per 100 ml of water (Blumenthal et al, 2000). Reclaimed water is usually treated and because it likely originated from human sewage sources, can still contain human pathogens (Sadovski et al, 1978; Bastos and Mara, 1995; Oron et al, 2010). Treatment of reclaimed water may vary by municipality and location, so the quality of reclaimed water may not be consistent and could easily exceed microbial counts found in surface water sources. Conserving drinking water sources and using surface water sources responsibly is important to the management of water as a natural resource and should be a consideration for growers in assessing the risks of using surface water in relationship to other management issues.

Key Extension Points

This project provided several insights into irrigation water quality and on-farm management practices related to water use. First, it was noted that on-farm water testing is not the standard practice on NY farms. With the increased pressure from buyers and the impending federal produce regulation, interest in water testing is increasing, but much extension work needs to be performed to provide adequate training for farmers. Training should include water testing protocols and how to monitor surface water sources through environmental assessment and analysis of water testing results. Growers will need to implement water testing practices for the surface water sources they are using and conduct a risk assessment of their water use practices implementing steps that reduce any identified risks. It is hoped that new research will continue to provide better data that will allow growers to make decisions that are as science-based as possible since current water testing recommendations vary in terms of testing frequency and acceptable quality limits. Data from this project indicates that surface water quality can vary dramatically over the season and there are not always clear factors to indicate why these

variations occur. Although this project only sample on-farm water sources three times during each growing season, it may be more practical to recommend that growers test their surface water sources prior to the start of season and *at least* once a month during use or more often if there are concerns about the quality of the water. Testing should be targeted at times just prior to use so they have an understanding of quality prior to the application, particularly if the water application is close to harvest. Reviewing water testing results on a per farm basis and incorporating other available information such as rainfall data will allow growers to gain valuable information in assessing the risks their surface water sources may represent.

To implement water testing practices, growers will need access to water testing laboratories that can provide testing of surface water sources. Not every commercial water testing laboratory may be prepared to handle surface water samples and provide the type of testing services farmers may need and request. Water samples also require analysis within a certain period of time. Some protocols state within 8 hours while others allow up to 30 hours of hold time prior to analysis. This is important because some farms are not located near water testing laboratories and so they will be forced to use overnight mail delivery for sample submission and would not meet the 8 hour requirement. Research has indicated that as long as the samples are kept at or below 10°C and not frozen, a 48 hour analysis window results in comparable data generation (Pope et al, 2003). If water testing becomes required, it is important that farmers be provided with a standard they can meet and that the resources such as access to water testing laboratories are available.

Limitations of this Project

In evaluating this project, several things became apparent that limited the ability to analyze certain data sets. First, the collaboration required to sample water throughout NY over a

two year time frame resulted in inconsistent sampling procedures. For instance, when sampling from irrigation equipment, individuals taking samples were instructed to sample the source water as well so that comparisons could be made. This did not always happen, and in a few instances, this resulted in a limited ability to fully explain sample anomalies. Another unfortunate lack of data collection was that in year one coliform counts were not recorded even though the water testing produced this information. One of the reasons this was not identified initially, was due to the compression of the sampling season because of the late arrival of research funds from the funding agency. In addition, sample analysis time was not recorded in the first year but was added in the second year. Sixty five percent (109) of the samples in 2010 were processed within 24 hours with all but one of the remaining samples (58) processed within 30 hours.

This late arrival of research funds also resulted in inconsistent sampling throughout the two seasons. Season one sampling happened very late in the season because the research funds were transferred late. Season two sampling started at the beginning of the season due to on-time arrival of research funds. It would have been preferable to have the sampling seasons overlap more thoroughly.

Another data point that would have been relevant but was not collected was the temperature of the sample at arrival. This information was deemed not relevant because it would not reflect the temperature of the water source, but it would have been relevant as a data point regarding data analysis. All water samples were shipped with sufficient ice packs and the receiving laboratory was monitoring the sample arrival to make sure the samples arrived cold, but it would have been good to have the data to verify the arrival temperatures.

A persistent unknown in each growing season was the weather. Initially, it was planned that all water samples would be taken from irrigation equipment. Data analysis for this project

indicates that this would not have been a good thing, so it was with fortunate luck that the first sampling season had sufficient rainfall and very little use of irrigation equipment by participating farms so most samples were taken from irrigation source water. This is a key point for future projects, that sampling source water is important especially if the project includes sampling from irrigation equipment.

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Appendix 3–A



The National GAPs Program

Water Quality Database

Data Entry Instructions

1. Launch your web browser (Internet Explorer, Firefox, Safari).
2. In the address line of your browser, enter the following web address: <http://fm31.888.net>. You will be presented with the following screen:

Instant Web Publishing

Enter an account name and password to view databases hosted by FileMaker Server:

Guest Account

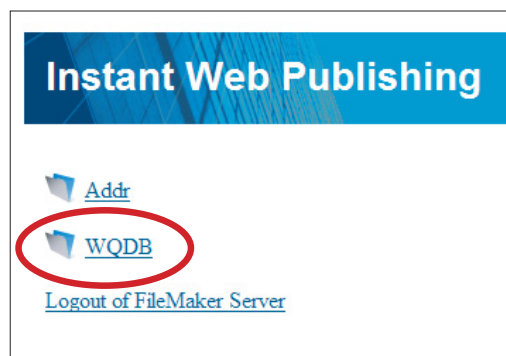
Account Name and Password

Account Name:

Password:

Login

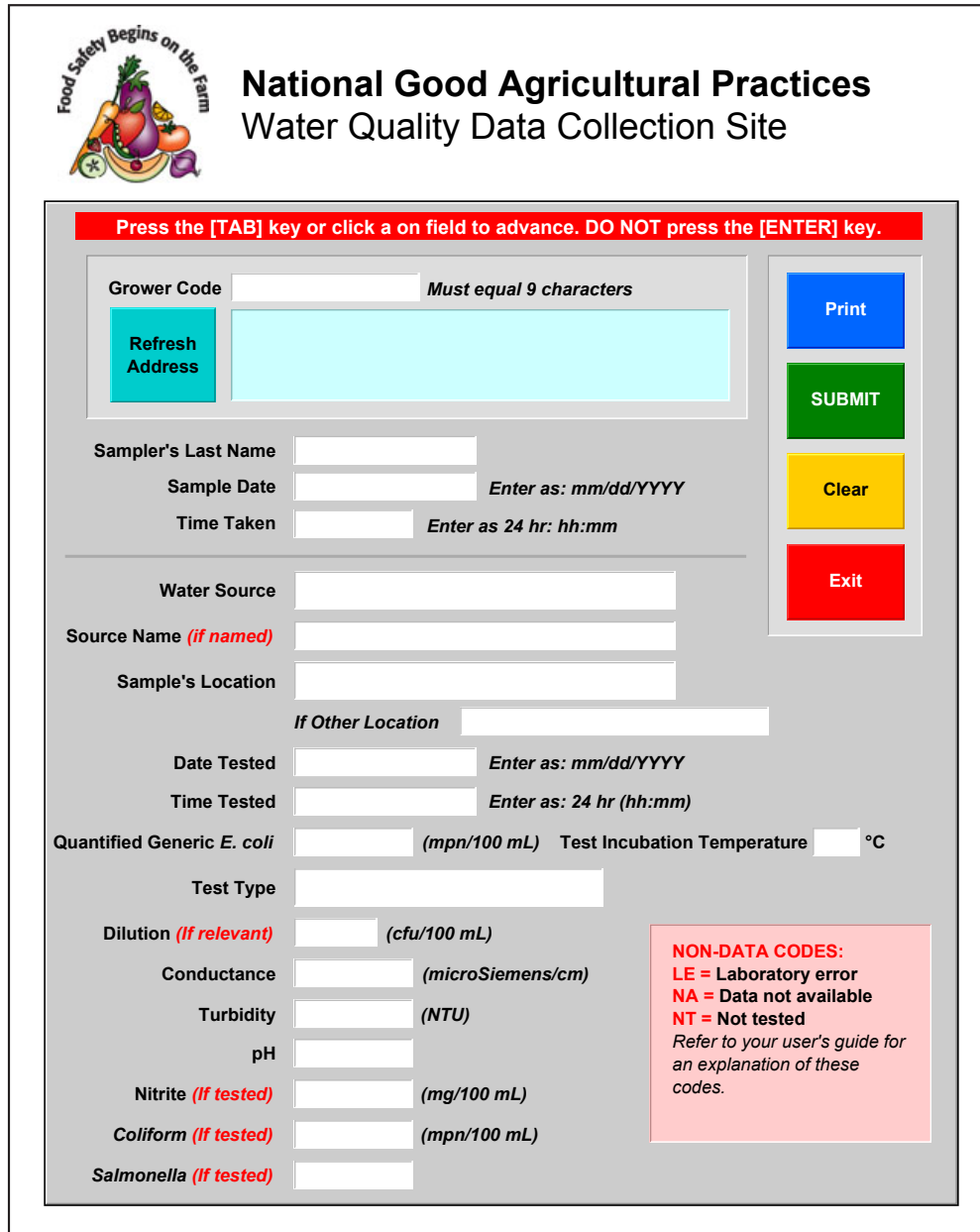
3. Make sure that “Account Name and Password” is selected. Enter your assigned Account Name (not case sensitive) and Password (**case sensitive**). Click on the “Login” button.
4. Upon successful login, you will be presented with the following screen:



5. Click on the file named “WQDB”. This is the file that you will be entering data into. The file labeled “Addr” is an address file that the WQDB file references when printing the grower’s report.

6. After you have successfully logged in, you will be presented with the following data entry screen:

IMPORTANT: When entering data, please press the [TAB] key to advance to the next field. **DO NOT** press the [ENTER] key. Pressing the [ENTER] key will add a carriage return to a data field which will return an error message upon processing.



Food Safety Begins on the Farm

National Good Agricultural Practices Water Quality Data Collection Site

Press the [TAB] key or click a on field to advance. DO NOT press the [ENTER] key.

Grower Code *Must equal 9 characters*

Refresh Address

Print

SUBMIT

Sampler's Last Name

Sample Date *Enter as: mm/dd/YYYY*

Time Taken *Enter as 24 hr: hh:mm*

Clear

Water Source

Source Name *(if named)*

Sample's Location

If Other Location

Date Tested *Enter as: mm/dd/YYYY*

Time Tested *Enter as: 24 hr (hh:mm)*

Quantified Generic *E. coli* *(mpn/100 mL)* Test Incubation Temperature °C

Test Type

Dilution *(if relevant)* *(cfu/100 mL)*

Conductance *(microSiemens/cm)*

Turbidity *(NTU)*

pH

Nitrite *(if tested)* *(mg/100 mL)*

Coliform *(if tested)* *(mpn/100 mL)*

Salmonella *(if tested)*

Exit


NON-DATA CODES:
LE = Laboratory error
NA = Data not available
NT = Not tested
Refer to your user's guide for an explanation of these codes.

7. Enter the grower code that appears on the upper right-hand corner of the “Water Sample Collection Form”. *In the event the cursor does not appear in the “Grower Code” field and you cannot click inside it, click on the “Sampler’s Name” field, hold down the “Shift” key and press the “Tab” key once. This will tab you backwards to the “Grower Code” field.*

8. After you have entered the grower code, click on the “Refresh Address” button. The name and address associated with this grower code will appear in the light blue box below the Grower Code field. The name in this field **MUST** match the name on the collection form. **The Grower Code must be EXACTLY nine characters in length, otherwise the grower address information will not appear.**


9. After all required fields have been entered, click on the “Print” button located at the upper right-hand corner of your screen.

10. After you click “Print” you will be directed to the grower report print screen. Select “Print” from your **web browser’s** print menu. After you have printed your document, click on the “Return” button to return to the data entry page.



National Good Agricultural Practices Water Collection Grower Report

This report was prepared for:
John Doe
123 Main St.
Anytown, New York 12345



Sample Date: 4/2/2010

Sample Received: 4/2/2010

Water Source: Pond

Sample's Location: Overhead irrigation


Source Name:

Quantified Generic *E. coli*: 110 mpn/100 mL

***E. coli* Temperature:** 35° C

Test Type: Coli-lert Quantitray mpn/100 mL	Dilution (if relevant):
Conductance: 1800 microSiemens/cm	Nitrite (If tested):
Turbidity: 20 NTU	Coliform (If tested):
pH: 7.4	Salmonella (If tested):

NOTE: All fields are required except for those indicated below. All non-required fields may be left blank.



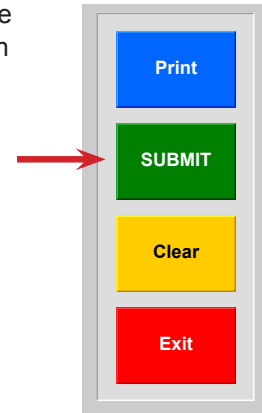
National Good Agricultural Practices Water Quality Data Collection Site

Press the [TAB] key or click a on field to advance. DO NOT press the [ENTER] key.

Grower Code	<input type="text" value="461000013"/> <i>Must equal 9 characters</i>	<input type="button" value="Print"/> <input type="button" value="SUBMIT"/> <input type="button" value="Clear"/> <input type="button" value="Exit"/>
<input type="button" value="Refresh Address"/>	<input type="text" value="John Doe"/> <input type="text" value="123 Main St."/> <input type="text" value="Anytown, New York 12345"/>	
Sampler's Last Name	<input type="text" value="Smith"/>	
Sample Date	<input type="text" value="4/2/2010"/> <i>Enter as: mm/dd/YYYY</i>	
Time Taken	<input type="text" value="13:30"/> <i>Enter as 24 hr: hh:mm</i>	
Water Source	<input type="text" value="Pond"/>	
Source Name (if named)	<input type="text"/>	This field is not required.
Sample's Location	<input type="text" value="Overhead irrigation"/>	
	<i>If Other Location</i> <input type="text"/>	
Date Tested	<input type="text" value="4/3/2010"/> <i>Enter as: mm/dd/YYYY</i>	
Time Tested	<input type="text" value="9:00"/> <i>Enter as: 24 hr (hh:mm)</i>	
Quantified Generic E. coli	<input type="text" value="110"/> (mpn/100 mL) Test Incubation Temperature <input type="text" value="35"/> °C	
Test Type	<input type="text" value="Coli-lert Quantitray mpn/100ml"/>	
Dilution (if relevant)	<input type="text"/> (cfu/100 mL)	<p style="color: red; font-weight: bold;">NON-DATA CODES:</p> <p style="color: red; font-weight: bold;">LE = Laboratory error</p> <p style="color: red; font-weight: bold;">NA = Data not available</p> <div style="border: 2px solid red; padding: 5px; margin-top: 10px;"> <p style="text-align: center;">These fields are not required.</p> </div>
Conductance	<input type="text" value="1800"/> (microSiemens/cm)	
Turbidity	<input type="text" value="20"/> (NTU)	
pH	<input type="text" value="7.4"/>	
Nitrite (if tested)	<input type="text"/> (mg/100 mL)	
Coliform (if tested)	<input type="text"/> (mpn/100 mL)	
Salmonella (if tested)	<input type="text"/>	

11. After you have entered all of the required data for each grower, click on the “Submit” button. Your data will be submitted and you will be presented with a new, blank data entry screen for the next water sample collection form.

You may be provided with more than one collection form for an individual grower. Each form **MUST** be entered and submitted individually into the database.



NOTE: All fields are required except where noted (see page 5). The following list of codes are acceptable values in all fields except for the “Grower Code” field:

“LE” = **Laboratory Error** — Data loss in the lab for any given test.

“NA” = **Not Available** — Water sample collection form is missing information: Sampler’s Last Name, Collection Date, Time Collected, and/or Sample’s Location.

“NT” = **Not Tested** — Item was not tested.

The fields: “Water Source”, “Sample Location” and “Test Type” have one of these codes appropriately incorporated in their respective drop-down lists.

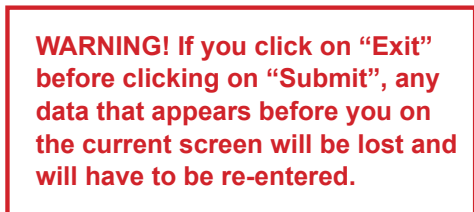
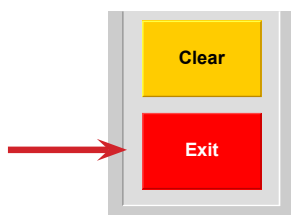
In the event that you did not fill in all required fields **OR** the grower code you entered does not equal 9 characters, **OR** you pressed the [ENTER] key in any field, you will see the following message upon clicking the “Submit” button:



Click anywhere on the red box and you will return to the data entry page. Review your data and make the necessary corrections and click on the “Submit” button again. If you are unable to find the error in any of the fields, press the “Clear” button. The “Clear” button will remove the data you entered from all fields. **Upon clicking on the “Clear” button, all field data will have to be re-entered.**



12. After you have completed data entry of all available water sample collection forms, you may exit the database by clicking on “Exit”. This will ensure that you are properly logged out of the database.



Appendix 3-B

Water Sampling Protocol

Equipment:

Marker to label bottles
Water sampling stick (not required but helpful)
Disposable gloves
Sampling container (1 Liter bottle)
Cooler
Ice packs
Shipping labels (if mailing to lab)
Tape
Ziplock Bags
Garbage/disposal bag for waste

Identify Water Testing Laboratory

Find a laboratory that is capable of providing the analysis you need. The National GAPs Program currently recommends testing for quantified generic *E.coli*. Tests that can achieve this type of analysis include Colilert Quantitray 2000 and modified mTec (EPA 1603). There may be others types of tests that can be used, but be certain to specify what you are looking for and the type of water source since many labs are not prepared to handle surface water sources. For a list of laboratories in New York State, visit the National GAPs Program website at www.gaps.cornell.edu.

The following sampling guidelines are recommendations. If the lab specifies a protocol or sampling container requirement, follow those recommendations since they may be required for the type of analysis they use.

Sample Collection

Write the date, time, and collection site (source, irrigation equipment) on the sampling container. Verify that this information is correct before leaving the sampling site.

Collect water sample as close to point of use as possible. This can mean from irrigation equipment in the field or in the water source, close to where irrigation equipment draws the water. Select a spot that is not filled with dense vegetation or litter, where you can collect a sample of representative water. If available, use a sampling stick. Place bottle on sampling stick and secure with strap. If you do not have a sampling stick, find a water access point where you will not disturb the bottom sediment.

Place clean gloves on your hands. While wearing gloves, carefully remove the lid of the bottle making certain to not stick your fingers inside the clean bottle or on the rim. Keeping the bottle clean will ensure a good sample collection. Extend the sampling stick out into the surface water source and capture a 1 liter sample. Do not disturb bottom sediments. If the surface water

source is very shallow, this may be a challenge. If the sample gets contaminated with bottom sediments, discard the sample. Use a clean bottle and sample in another location that has not been disturbed. Place the lid back on the bottle and tighten. Check to ensure cap is tightened on bottle. Place samples on ice within 15 minutes of collection.

Delivering Sample to the Laboratory

All samples should be delivered to the selected lab on the day of collection or shipped overnight early delivery. If shipping bottles, you may want to place the liter bottle in a ziplock bag and pack it snugly in the box with ice and packing peanuts. If shipping more than one location to the lab in one day in the same box, be extra careful that all samples are properly labeled so that there is no confusion about the origin of the sample. Ship samples with plenty of ice.

Water Sampling Protocol for Surface Water- Summary

Collection of Water Sample

*Always follow instructions provided by selected lab regarding container and sampling protocol.

1. Label bottle with name, water source type, date, and time of collection
2. Identify good sampling area, sampling nearest use area as possible
3. Assemble bottle on sampling stick if using a sampling stick
4. Put on gloves
5. Uncap bottle as close to the water source or irrigation equipment as possible. Do not place fingers on bottle lip or inside bottle.
6. Dip bottle into source and collect water. If sampling from irrigation equipment, do not let bottle lip contact irrigation equipment. Collect 1 L sample from each location
7. When bottle is full, tightly cap. Be sure to not touch the inside of the bottle of the lip.
8. Double check bottle labeling to be sure it is correct.
9. Place the water bottle in 1 gallon ziplock bag and seal
10. Place in cooler with ice packs.
11. When done sampling, label cooler and seal
12. Deliver to selected lab or drop at shipping company for shipment

Chapter 4

Assessing the Presence of *Salmonella* spp. in Surface Water Sources Utilized in the Production of Fresh Fruits and Vegetables

Introduction

Foodborne diseases from both known and unidentified pathogens are estimated to cause approximately 47.8 million illnesses in the United States each year (Scallan et al, 2011b). Some foodborne illnesses are so severe they result in death. Four pathogens, *Salmonella* spp., *Toxoplasma gondii*, *Listeria monocytogenes*, and norovirus, account for approximately 82% of deaths that result from foodborne illnesses by major pathogens in the United States (Scallan et al, 2011a). This reflects risks from consuming all types of foods, with no single food group isolated. When foodborne illness data related to the consumption of fresh produce is reviewed, the importance of *Salmonella* as a foodborne pathogen is once again highlighted. From 1996-2007, there were no produce-associated foodborne illness outbreaks reported that were caused by norovirus, *Toxoplasma* or *Listeria*, but 28 of 72 (39%) reported outbreaks were caused by *Salmonella* spp. (Vierk, 2008). *E. coli* O157:H7 and *Cyclospora* accounted for 29% and 22% of the outbreaks, respectively. *Salmonella* spp. not only accounted for the most outbreaks, but also the most deaths, though *Cyclospora* caused the most illnesses (Vierk, 2008). In the recently released document entitled *Ranking the Risks: The 10 Pathogen-Food Combinations with the Greatest Burden on Public Health* by Batz et al (2011), the pairing of *Salmonella* and produce ranked number eight in terms of annual disease burden.

Contamination of fresh fruits and vegetables with pathogens can occur anywhere in the supply chain from field to fork. Potential contamination can result from contact with the soil,

manure, water, animals, workers, equipment, transportation vehicles, improper storage, packaging, display, and preparation (Beuchat and Ryu, 1997; CDC, 1997; Brackett, 1999; CDC, 2002; CDC, 2003; Bihn and Gravani, 2006; Doyle and Erickson, 2008). Fresh fruits and vegetables grow in the farm environment where *Salmonella* and other human pathogens have been found in the soil, water, wildlife, and in airborne particles (Sargeant et al, 1999; Steele and Odumera, 2004; Gerba and Smith, 2005; Beuchat, 2002; Brandl, 2006; Hutchison et al, 2008). Subsequent handling steps required to harvest, pack, transport, and market fresh produce increase the opportunities for contamination and even growth of pathogens that may be present.

Food safety begins on the farm and so preventing contamination of fresh produce by microbial pathogens during production, harvest and packing is important. Eliminating all risks on the farm is not possible but reducing risks is certainly achievable and has been the focus of many government and university extension programs, as well as commodity organizations (USFDA, 1998; Rangarajan et al, 2003; FDACS, 2007; CSFSGPHLLG, 2010). Any agricultural management practices or operational procedures that reduce microbial risks or prevent contamination of fruits and vegetables on farms or in on-farm packinghouses are referred to as Good Agricultural Practices (GAPs). GAPs encompass a wide variety of food safety practices and provide latitude so that every fresh produce grower can implement the practices that are most effective and efficient at minimizing the risks that exist in their operations. This is necessary because each operation is unique and production practices may differ depending on cultural practices, location, and commodities grown. To optimize the time and resources committed to implementing food safety practices, growers should conduct a risk assessment and target the implementation of GAPs that reduce identified risks.

One method of assessing risks is through monitoring programs. Monitoring workers and their hygiene practices, wildlife access to fields, and the quality of surface water used during production are just a few examples of common monitoring programs. Some monitoring programs have very prescribed practices such as those targeted at water quality monitoring in the Tomato Best Practices Manual and the Commodity Specific Food Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens (FDACS, 2007; CSFSGPHLLG, 2010). Both of these programs outline sampling frequencies and required testing for quantified generic *E. coli* for surface water sources used during fresh produce production as a way to monitor surface water quality. The presence of generic *E. coli* is an indicator of fecal contamination and represents the potential for other fecal borne pathogens to be present. Surface water does not have to be free from generic *E. coli* contamination to be used, but monitoring the water quality allows growers to make informed decisions about the use of the water in question. Unfortunately, testing for generic *E. coli* will not provide an accurate assessment of the presence or absence of *Salmonella* spp. or other foodborne pathogens. Since *Salmonella* spp. cause many foodborne illness outbreaks, and has an identified relationship with fresh produce, a survey to assess the presence of *Salmonella* spp. in surface waters intended for use during the production of fresh produce was conducted.

Materials and Methods

Sample Water Collection

One liter of water was collected from each sample site into bottles, either cleaned or purchased following a protocol that resulted in decontamination of the bottles (EPA protocol “B”). Collected samples were placed in a cooler with ice packs and delivered directly to the laboratory or sent via overnight delivery to a laboratory for analysis (NYS Food Laboratory or

Cornell laboratory). Samples were analyzed for *Salmonella*, generic *E. coli*, specific conductance, pH, and turbidity. Samples were stored at 4°C and analyzed within 24 hours of sampling. All *Salmonella* analysis was completed in Dr. R. Worobo's and Dr. C. Weber's laboratories at Cornell University.

Selective Media Screening and Enrichment for Salmonella spp.

Several methods were utilized for screening samples for *Salmonella* including direct plating of surface water onto selective media, membrane filtration, and enrichment. For direct spreading, 100 µl of the surface water was plated directly onto Difco Bismuth Sulfite (BS) plates (Franklin Lakes, NJ) using aseptic techniques. In addition to direct spreading, 25-100 mls of surface water (depending on turbidity of the water) was filtered through Millipore 0.22 µm nitrocellulose filter (Billerica, MA) and placed directly onto BS plates. Both the direct spread plate and the filter plate were incubated at 37°C for 24 hours. Plates were evaluated for presumptive positive *Salmonella* colonies (black/green metallic colonies). Presumptive *Salmonella* colonies were sub-cultured onto Brain Heart Infusion (BHI) agar in preparation for Polymerase Chain Reaction (PCR). In addition to screening on BS plates, Difco Xylose Lysine Deoxycholate (XLD) plates were added to the protocol for analysis mid-way through the experiment to provide parallel analysis to the enrichment protocol.

For enrichments, 100 mls of each sample were filtered through 0.22 µm nitrocellulose filter (Millipore). The filters were placed in Whirlpak™ bags with 90 mls of peptone trypticase soy broth (pTSB) for 2 hours at 24°C, then up to 24 hours at 37°C. To evaluate the impact of the pre-enrichment step, one set of samples was pre-enriched with both pTSP and phosphate trypticase soy broth, in parallel. Pre-enrichment cultures were then sub-cultured into two

different selective enrichment medias including Oxoid Rappaport Vasiliadis (RV) broth (Lenexa, KS) and Difco Tetrathionate (TT) broth then incubated for 24 hours at 42°C in a shaking water bath. After incubation, 50 µl from each enrichment was plated onto Difco Xylose Lysine Deoxycholate (XLD) plates and incubated at 37°C for 24 hours. Enrichments were also plated onto BD BBL® CHROMagar® Salmonella plates (Franklin Lakes, NJ) when available. XLD and CHROMagar® plates were evaluated and presumptive positive colonies were streaked onto BHI plates. Atypical colonies were selected from plates that had no presumptive positive colonies, as per the suggested protocol in the FDA Bacteriological Analytical Manual (Andrews et al, 1995). Presumptive positive and atypical colonies were analyzed for confirmation by PCR analysis.

Preparation of DNA and PCR Assay parameters

Individual colonies were taken directly from the different selective media using sterile toothpicks and placed in a sterile microcentrifuge tube with 100 µl of sterile distilled water, and then microwaved (1200W Panasonic) for 30 seconds to lyse the cells by boiling. Samples were cooled on ice, and then stored at 4°C until use. Nucleic acid concentration of each sample was determined through spectrophotometric quantification. Two *Salmonella* controls were used (FSL S5-370, Wiedmann Lab, Cornell University; ATCC 14028, Dr. R. Worobo, Cornell University). The 25 µl PCR reactions included 15.7 µl sterile dH₂O, 2.5 µl of 5x buffer (49.3 mM Tris-HCl, 2.5mM MgCl₂, 1 mM tartrazine , 1.5% ficoll), 0.5 µl dNTP (10 mM), 0.5 µl of each primer (10 µM), and 0.3 µl Taq polymerase, and 5 µl DNA preparation (25 ng/µl). The PCR was run on a MJ Research PTC-100 Thermal Cycler (Waltham, MA) under the following conditions: denaturation at 94°C for 2 minutes (1 cycle); denaturation at 94°C for 45 seconds (s), primer annealing at 60°C for 30 s, and extension at 72°C for 45 s (35 cycles); final extension at

72°C for 5 minutes (1 cycle); sample hold at 4°C. A 15 µl aliquot of the reaction mixture was electrophoresed on a 1.5% agarose gel in a Tris-acetate-EDTA (TAE) buffer. Amplified products were stained with ethidium bromide and visualized by UV transillumination.

The oligonucleotide primers for PCR were synthesized according to the published DNA sequences for *invA* gene of *Salmonella* spp. (Chiu and Ou, 1996; Kim et al, 2007) (Table 4-1). The *invA* gene sequence can be used in PCR to specifically identify *Salmonella* spp. (Rahn et al, 1992). *Salmonella* is a facultative intracellular pathogen and its *invA* gene encodes for proteins that facilitate cell invasion (Galan et al, 1992). The *invA* primer set that resulted in a 243 base pair (bp) product is referred to as primer set (PS) 1 and the *invA* primer set that resulted in a 678 bp product is referred to as PS 2. A 16s rRNA oligonucleotide primer set was included to confirm the presence of amplifiable DNA in each sample that was analyzed (Edwards et al, 1989).

Table 4-1. Oligonucleotide primers used for PCR

Bacterium	Primer	Sequence (5' to 3')	Target gene	Product (bp)
<i>Salmonella</i>	Forward	ACAGTGCTCGTTTACGACCTGAAT	<i>invA</i>	243
	Reverse	AGACGACTGGTACTGATCGATAAT		
<i>Salmonella</i>	Forward	GAATCCTCAGTTTTTCAACGTTTC	<i>invA</i>	678
	Reverse	TAGCCGTAACAACCAATACAAATG		
Eubacteria	Forward	AGAGTTTGATCCTGGCTCAG	16s rRNA	1534
	Reverse	AAGGAGGTGATCCAGCCGCA		

Results and Discussion

PCR

Thirty one on-farm surface water sources on fruit and vegetable farms in New York State were sampled in the fall of 2010 and surveyed for the presence of *Salmonella* spp. Several presumptive positive and atypical colonies were selected from the direct plating, as well as the enrichment selective process. PCR analysis was performed on 65 DNA isolates resulting from these colonies and two *Salmonella* controls. Figure 4-1 shows a representative collection of outcomes from the unknown DNA isolates from on-farm surface water sources and resulting PCR products when using either the 16s rRNA primer set or PS 2. The 16s rRNA primer set verified the presence of amplifiable DNA resulting in a 1534 bp product. No positive *Salmonella* spp. colonies were identified from the on-farm surface water sources using either the PS 1 (data not shown) or PS 2. Figure 4-2 includes the resulting PCR products when *Salmonella* control DNA is amplified using PS 2 (A) and PS 1 (B). PS 2 resulted in a 678 bp product and PS 1 resulted in a 243 bp product.

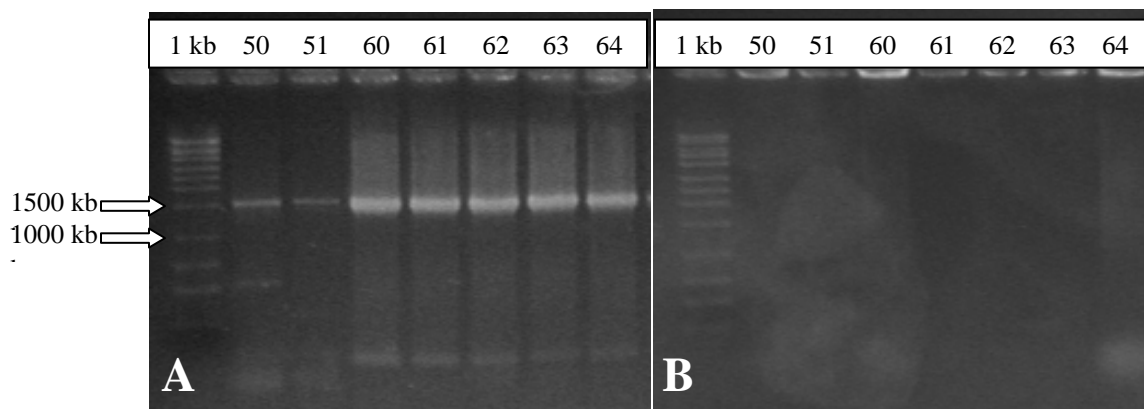


Figure 4-1. Image A is a representative collection of PCR products from DNA isolates from on-farm surface water samples using 16s rRNA primers resulting in a 1534 bp product. Image B is the same samples using PS 2 in the PCR reaction. No PCR product of expected size resulted, indicating that the samples are negative for the presence of *Salmonella* spp.

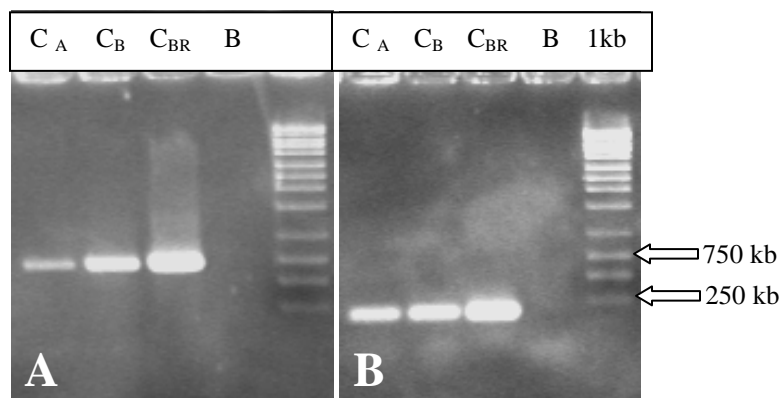


Figure 4-2. Image A depicts the PCR product resulting from amplification using PS 2 with the *Salmonella* controls. C_A is *Salmonella* control (Dr. Wiedmann's laboratory), C_B is *Salmonella* control (Dr. Worobo's laboratory), C_{BR} is control C_B that was boiled twice, and B represents a blank control. Image B depicts the PCR product resulting from amplification using PS 1 with the *Salmonella* controls.

Experimental Observations

Only one location that was sampled resulted in typical presumptive positive colonies on the selective medias. This location had presumptive positives on both the direct spread plates and the colonies resulting from enrichment. From all other sources, atypical colonies were chosen for analysis. Neither typical nor atypical colonies resulted in expected size PCR products when amplified with *invA* specific primers, indicating that all on-farm surface water sources surveyed were negative for *Salmonella* spp. This does not mean there was no *Salmonella* present, but that this particular experiment did not detect any using the volumes of waters sampled. This highlights some of the commonly encountered problems when looking for pathogens; they are often difficult to detect, below detectable levels or may be injured (Riordan et al, 2001). At the time of sampling, each surface water source that was collected was also analyzed for total coliforms and generic *E. coli*. The 31 on-farm water sources ranged from 93.2 to >2419.6 Most Probable Number (MPN)/ 100 mls total coliforms and <1 to 166.4 MPN/ 100 mls generic *E. coli* (geometric mean = 10 MPN/100 mls), so there was clearly microbial

populations present in the water sources. The one water source that resulted in typical presumptive positive colonies of *Salmonella* had 770.1 and 10.9 MPN/100 mls total coliforms and generic *E. coli*, respectively. Although these colonies were not confirmed through PCR amplification with *Salmonella* gene specific primers, it is interesting to note that this water source did not have the highest microbial counts using total coliforms and generic *E. coli* as indicators.

In comparison to other reports of surface water microbiology, this indicates that the level of *E. coli* and *Salmonella* spp. are lower than other produce production regions relying on surface water (Duffy et al, 2005). There may be several reasons for this result including lower incidence of waterfowl, amphibians, or reptiles present on NY farms tested or due to seasonal variations. The primary animal pressures on the NY farms tested were dairy cattle and deer with no large scale poultry farming operations in the vicinity of the surface waters tested, though there were wild bird populations such as ducks and geese in close proximity to the water sources. In states outside of NY where poultry farming is more predominant, there may be higher potential for *Salmonella* spp. in surface waters (Mallinson et al, 2001). In an additional study that was conducted (results unpublished), the incidence of *Salmonella* spp. isolated from surface waters was highly variable depending on the season and water source, with spikes in June and late September. The presence of *Salmonella* spp. was not dependent on the presence of *E. coli*.

One modification that could be made would be to sample a larger volume of water at each source. There are challenges that are associated with increasing the volume of water sampled because surface waters are often turbid making filtration difficult or background microflora may mask the identification of specific pathogens of interest. Additional volumes could be filtered using traditional methods by increasing the number of filters if turbidity causes

problems or through the use of new capture devices that rely on higher surface area membranes but with higher molecular weight cut offs (T. Suslow, UC Davis; M. Danyluk, U of Florida; personal communication). As sampling techniques allow for filtration of larger volumes, it will allow for a better assessment of surface water sources. From a research perspective, this will be a significant advance for investigating the presence of *Salmonella* spp. in on-farm water sources, but it seems unlikely that this approach will be widely available or financially feasible for many growers.

Since *Salmonella* contamination of fresh produce represents such a significant food safety risk, it would be valuable to invest research efforts into determining the most efficient and effective way to test on-farm water sources while remembering that the method must be easily adopted by commercial laboratories and inexpensive to perform. Currently, generic *E. coli* is used as the indicator of fecal contamination and the potential presence of fecal associated foodborne pathogens (Tortorello, 2003). A more reliable indicator microorganism for irrigation water and produce sampling would greatly facilitate the microbiological assessment of surface waters intended for fresh produce production.

Developing effective and affordable testing strategies for organisms that reliably indicate the quality of surface water used for fresh fruit and vegetable production is important, but there is still another area that requires significant research. Understanding the risk that contaminated water poses to the safety of fresh produce is still poorly understood and is critically important to on-farm decision making regarding surface water use during fresh produce production. Many have reported the lack of correlation between irrigating with water that contains contamination and finding that contamination on commodities irrigated with that water (Riordan et al, 2001; Zhou et al, 2005; Bihn, unpublished data). It is understood that the timing of water applications

and field environmental conditions including UV radiation and desiccation can reduce risks from surface water (Steele and Odumeru, 2004; Hutchison et al, 2008; Wood et al, 2010) but currently there is no standardized, scientifically verified, surface water application protocol that addresses risks based on the quality of water applied or the commodity to which it is applied. This type of research is very difficult because of the variable quality of surface water and environmental conditions. Given limited research funds, it would be justified to review risk assessment data based on commodities and pathogens and focus limited resources on reducing risks in areas where they are most likely to have an impact on human health.

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Chapter 5

Prospectus

Risks Assessment and the Implementation of Good Agricultural Practices

A significant challenge to the implementation of Good Agricultural Practices (GAPs) on every farm in the United States (US) is the need for each farmer to have a working knowledge of biological, chemical, and physical hazards that affect the crops that they grow, harvest, pack, and sell. This knowledge is needed so that they are able to identify risks in their own operations and implement the appropriate food safety practices such as GAPs to reduce the identified risks. In every state across the US, there are many farmers who grow fresh fruits and vegetables and sell them, whether through wholesale markets or directly to consumers. Reducing the risks to fresh produce during production will require reaching all fruit and vegetable farmers and providing them with the educational resources necessary to enable them to assess the food safety risks that exist in their operations. In addition, farmers will have to be committed to the process of risk reduction because GAPs implementation will require them to commit financial and human resources to the effort. This represents an opportunity for university extension professionals and others who work directly with farmers to integrate current research into practical solutions.

The research generated and discussed in this dissertation is one example of research that can improve risk assessment and the practical implementation of GAPs to reduce risks on the farm. Understanding the quality of surface water and factors that impact the likelihood of contamination are important for assessing risks on farms that use surface water for fresh produce production. Currently, some commodity groups are using the EPA recreational water standards as a standard for determining when surface water is safe to use for production of fruits and

vegetables. These standards require that decisions be made on the geometric mean of a collection of water samples. Based on data analysis in this dissertation and the desire to provide farmers with the most practical way to understand risks, it may be more appropriate to use the median as a calculation across several water samples as opposed to geometric means. In side by side calculations, the median calculation aligns well with geometric mean calculations but is much easier to calculate and understand. Geometric means require at least five water samples to be meaningful and with less than five, the results can be undependable. Using the median could have similar dependability issues but determining the median is much more straight forward and would allow the growers to see the wide variation if it exists. Alternatively, the arithmetic mean could be an option, but typically in the data sets presented in this dissertation, the arithmetic mean was much higher than the median or geometric mean. The geometric mean also helps to minimize the impact of outlying data points, but it is not clear that this is a good idea if the outlying data is a very high water test result during a time when farmers plan to use the water, as it may be an indication of risk. Using calculations that are easy to complete and understand is critical to functional risk assessment and for these reasons, perhaps it is time to use either median or arithmetic mean for the analysis of water testing data.

The food safety questions related to the use of surface water sources for the production of fruits and vegetables provide several opportunities for additional research. Due to the importance of *Salmonella* as a human pathogen and its association with fresh produce, more effort should be placed on understanding the routes of contamination and commodities that are most likely to become contaminated. In terms of surface water sampling, developing standardized methods for detection and enumeration are critical but so is the need to sample larger volumes of water. Identifying new indicator organisms that are easy to test for and

accurately reflect risks related to surface water would be very valuable to farmers and to the risk assessment process.

Developing the irrigation water quality database provided an example of how irrigation quality parameters from multiple states and regions can be collected and analyzed. It also highlighted many challenges that will continue to impede the collection of information related to irrigation water quality. An unwillingness among the produce industry and academic colleagues to collaborate and share data was a significant limitation to this project. Farmers also fear participation in projects that collect research data that could expose their farm to scrutiny by buyers or other farms that compete for their markets. Regardless of the challenges, collecting large data sets to obtain a true picture of environmental conditions and microbiological quality parameters is important to reaching science-based answers to produce safety practices. Expanding current databases is one way to improve the value of already existing information.

The origin of water used during fruit and vegetable production is another area that could be investigated to improve risk assessment. Some farms use reclaimed waste water to irrigate fresh produce and it seems likely this water may carry more human pathogens because of its origins in human sewage, though this specific research data is missing from the literature. The database that was developed as part of this dissertation could be expanded to include reclaimed water sources but it would also have to be expanded to include specific pathogen testing as simply testing for generic *E. coli* would not be sufficient to determine if reclaimed water truly represents a greater risk than water of other origins. There is sufficient flexibility in the database to make modifications to include new data and expand the scope of information collected.

Once risk assessments based on the best scientific information available have been completed, limited resources need to be targeted at implementing practices that reduce identified risks. A significant hurdle in focusing solely on risks from a farmers' perspective is managing buyers' demands. Food safety practices have always been market driven and even after the USFDA releases the upcoming produce regulation, it seems likely that buyers will still impact what food safety practices are implemented. Unfortunately, required food safety practices are not always based on addressing the risks but are based on one size fits all food safety requirements or audits. As an example, some very small farms are tasked with implementing visitor protocols and security policies that require significant human and financial resources to implement and manage record keeping. The risks related to food safety and food security at these operations related to visitors are miniscule, yet many are forced to spend limited resources implementing practices that do not address the highest risk areas.

Risk assessment and food safety practices implementation should also take into account the final use of the commodity. Many fruits and vegetables are destined to undergo processes that eliminate food safety risks. For example, potatoes destined to become potato chips that are processed in hot oil should focus food safety practices on reducing risks related to physical hazards such as rocks, not on microbiological risks that will be eliminated in the cooking process. This optimizes the use of resources including time and money which everyone can appreciate. Wasting time and money by imposing the implementation of practices that do not reduce risks increases frustration and results in less interest in effective practice implementation. Encouraging buyers to move away from one size fits all requirements may result in better risk reduction because limited resources can be spent on reducing real risks, not perceived risks.

There are several things that may encourage the acceptance of risk-based implementation of food safety practices and one factor currently in the market place is the demand for locally produced fruits and vegetables. Buyers want a local source of fresh produce and many local supplies come from small farms. Some buyers have been willing to work directly with these farmers and in some cases modify their one size fits all requirements to have small farms be their suppliers. Larger farm owners are aware of these disparities and see this as both unsafe and unfair because a produce associated foodborne illness outbreak will affect them proportionally more in the market place and lead to larger losses for them financially. This is another reason why emphasis should be put on science-based risk reduction since it provides a level of fairness in the market place for both small and large farms.

The federal government has prioritized the implementation of food safety practices on small farms through the establishment of the Produce Safety Alliance (PSA). Funded by the USDA and the USFDA, the PSA is charged with developing a national curriculum to help farmers understand and implement food safety practices such as GAPs on farms and in packinghouses with outreach to small-scale farms as a priority. At a moment in time when the USFDA is poised to release its first ever regulation regarding fresh produce safety, it is important to note that, that the US government does not have the resources necessary to enforce the regulation on every fresh produce farm in the US or on farms that import into the US. The Food Safety Modernization Act to some extent acknowledges this through the Tester Amendment that exempts farms that have under \$500,000 of sales from having to follow the regulation and therefore be subject to enforcement. The PSA provides funding to focus on science-based education and outreach that may have an impact on the practices required by buyers if they are included in the USFDA produce regulation and supported in the national curriculum.

The need for science-based risks assessment related to produce safety does not stop at the farm gate. Much emphasis has been placed on the farm in terms of produce safety but to truly impact safety and implement effective risk reduction strategies, the entire food system needs to be assessed. Packing facilities represent many opportunities for single contamination events to spread across many lots through improper disinfection of dump tank waters or improperly implemented sanitation programs. Retail markets have additional risks related to water, sanitation, and risks associated with consumers who handle produce they may not purchase. Once consumers make a selection and purchase fruits and vegetables, there are additional risks that are found during food storage and preparation in the home. Though this dissertation is focused on the farm, true risk reduction would benefit from increased communication throughout the food system.

Fruits and Vegetables: Sunny Side Up

An important point in any discussion about fresh produce or fresh produce safety is that eating fresh produce is **great** for you, not just good for you. In a country where obesity has a significant impact on health care costs due to increased chronic health disorders such as cardiovascular disease, type 2 diabetes, and high blood pressure, eating fresh produce offers a much needed healthy food option that is incredibly tasty. This is fantastic news for the fresh produce industry. Societal interest in local foods also is a good thing for the produce industry as a whole. Local fruits and vegetables provide people with the opportunity to purchase and consume produce at peak ripeness since it can be picked, marketed, and purchased within just a few days. Fresh, ripe, just picked produce is usually going to taste the best and result in people eating more fruits and vegetables. Once an individual becomes accustomed to eating produce,

they will continue to seek it out, which in upstate New York means sourcing fresh produce from retail markets that purchase fresh produce from other locations during the winter. More consumption is good for the industry because it increases demand and sales but it is also good for consumers because research indicates the more fresh produce an individual consumes, the more protection they gain against many chronic diseases.

The Achilles' heel of the fresh produce industry is produce associated foodborne illnesses. Foodborne outbreaks decrease immediate consumption and sales of commodities that are associated with the outbreak, but also have long-term impacts on production and consumption trends. Reducing the likelihood and number of produce-associated foodborne outbreaks is critical to both those who grow fresh produce and those that eat it. Research and extension programs that work with farmers to support the implementation of science-based practices that effectively reduce microbial hazards in fresh produce are good for consumers and the fresh produce industry.

The produce industry as a whole should spend time and effort promoting two key messages. The first is that there is no zero risk when it comes to the consumption of fresh fruits and vegetables, but that the fresh produce industry is actively engaged at all levels to provide the safest produce possible. The second message is that the health risks associated with not eating fresh produce are much larger than the risks of contracting a produce associated foodborne illness from eating fresh produce. As stated earlier, the benefits to human health of eating a variety of fruits and vegetables is scientifically supported through numerous nutrition and health studies. Fresh produce is also relatively low in calories, so for a country like the US that is battling an ever-increasing obesity epidemic, increasing fresh produce consumption could broadly impact the health of the nation and its medical costs. Encouraging the fresh produce

industry as a whole to promote the consumption of a wide variety of fresh produce will encourage increases in consumption of all fresh fruit and vegetable groups. The bar should be set at "More is better, but five is the minimum".

These two simple messages could have a significant impact on the industry. The message about no zero risk would allow a conversation to develop about the best way to invest limited farm resources to reduce actual risks, not the current shotgun approach of treating all risks with the same priority. Highlighting the positive health benefits of fresh produce consumption would encourage consumers to eat more fruits and vegetables. As part of this health message, the fresh produce industry should collaborate with nutritionist and culinary experts to provide easy to understand guides on what counts as a serving of fresh produce and how an average American can easily include more than five servings a day in their diet. They make simple feeding charts for new parents that outline proper nutrition for infants as they transition away from breast milk only diets. This same concept could be utilized to create consumption charts for fresh produce that include novel and tasteful ways to incorporate more fruits and vegetables into a daily diet. As someone working in the fields of food science and horticulture, I struggle to consume five or more servings of produce a day and in most cases, do not know what constitutes one serving for each of the commodities. These charts could be created, printed, or posted on websites with minimal effort and cost when shared across commodity groups. A unified marketing campaign across commodity groups would send a consistent message and lend credence to the importance of the message.

Farmers who produce fruits and vegetables care about produce safety from both a business perspective as well as a health perspective, because their own families eat what they grow. Reducing risks to fruits and vegetables through on-farm risk assessment and the

implementation of GAPs is achievable. It will take an investment in education and outreach, but farmers are ultimately capable of learning how to conduct risk assessments, developing strategies, and implementing practices that reduce risks. Clearly communicating the value and need to prioritize produce safety while providing science-based information will encourage farmers to engage in the process and commit resources to keeping fruits and vegetables safe.