PROJECT TITLE: ASSESSING AND REDUCING MICROBIAL CONTAMINATION IN VERMONT VEGETABLES

Co Principal Investigators:

<table>
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<tr>
<th>Name(s)</th>
<th>Department/Region</th>
<th>%Station Appointment*</th>
<th>% EXT Appointment**</th>
<th>%Effort</th>
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<tr>
<td>Vernon P. Grubinger</td>
<td>Southern Region Extension</td>
<td>0</td>
<td>100</td>
<td>5</td>
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<tr>
<td>Karen A. Schneider</td>
<td>Southern Region Extension</td>
<td>0</td>
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<tr>
<td>Milton E. Tignor</td>
<td>Plant and Soil Science Dept.</td>
<td>30</td>
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*VT-AES PI is responsible for all UVM and federal reporting (including the CRIS system) related to research activities.

**UVM Extension PI is responsible for all UVM and federal reporting related to extension activities.

PROJECT DURATION: 3 years

BUDGET REQUEST:

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TECHNICAL REVIEWERS:

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<th>Name</th>
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<tr>
<td>Dr. Anusuya Rangarajan</td>
<td>Assistant Professor, Fresh Market Vegetables Specialist, Department of Fruit and Vegetable Science, Cornell University, actively involved in food safety extension and research</td>
</tr>
<tr>
<td>Dr. Christopher Wilson</td>
<td>Assistant Professor, Agroecology, Indian River Research and Education Center, University of Florida, actively involved in research examining agricultural irrigation impacts on water quality</td>
</tr>
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</table>

Department Chair

Name: Alan Gotlieb
Signature:________________________Date:____________________

Regional Chair

Name: Neil Pelsue
Signature:________________________Date:____________________
JUSTIFICATION
Vegetable consumption is on the rise. Americans consumed 19% more fruits and vegetables in 1993 as compared to 1970 and vegetables are responsible for the majority of that increase from 1980 forward (U.S. Department of Agriculture, 1998). Additionally, the consumption of fresh, frozen, and dried vegetables, except tomatoes, has increased compared to canned counterparts (U.S. Department of Agriculture, 1998).

Vermont has over 300 vegetable farms that produce about 2900 acres of vegetables with an estimated market value of about 6.5 million dollars (U.S. Department of Agriculture, 1997). Interestingly, 67% of these farms produce less than 4.9 acres of vegetables and less than 3% produce more than 50 acres (U.S. Department of Agriculture, 1997). However, census data may be underestimating vegetable sales, given that a 1993 survey of Vermont growers found the value of vegetable production to be $8.6 million (Grubinger and DeGeuss, 1996). The same survey also found that 61% of vegetable and berry sales were direct sales to consumers. Successful entrepreneurs selling high quality produce to consumers via pick-your-own farms, roadside stands, and local farmers markets drive these intensive horticultural efforts.

Local retail marketing of vegetable produce has several advantages. Most importantly, the farmer earns a larger portion of the consumer food dollar compared to wholesaling. And, by establishing a direct relationship with the consumer, the farmer is somewhat insulated from fluctuations in wholesale vegetable prices. Small farms that direct-market their produce are often better able to monitor the quality of vegetables than are large distributors, and because small farms sell directly from the field to the consumer they can offer a fresher product. Small-scale production also lends itself to the utilization of a more diverse range of vegetable cultivars than large mechanized agricultural operations. Additionally, the diversity on many smaller vegetable farms facilitates the incorporation of organic and sustainable agricultural practices, such as crop rotation, into overall farm management schemes.

One potential problem of increasing public concern on small-scale vegetable farms in Vermont is contamination of water with microorganisms that pose a threat to food safety. On small farms, there are often more individual water sources (for irrigation and washing), organic fertilizer origins, and post-harvest facilities as compared to larger operations. For example, consider the additional number of potential point sources of contamination for twenty 5-acre farms versus a single 100-acre farm. Many vegetable farms use surface waters for irrigation, and because these waters have the potential to be impacted by livestock operations, particularly dairy, they are a potential source of microbial contamination. In addition, reliance on organic and sustainable production practices by Vermont’s vegetable farmers results in substantial handling and application of animal manures and composts, both of which are potential sources of microbial contamination.

Recent *E. coli* 0157:H7 outbreaks associated with fresh vegetables, such as the 1995 Peel Memorial Hospital and 1996 Fancy Cutt Farms lettuce incidents (as reviewed by (Anonymous, 1997)), have brought issues concerning microbial contamination in vegetables to the public’s attention. Annually in the United States, it is estimated that food borne illnesses cause 76 million cases of acute gastroenteritis, 325,000 hospitalizations, and 5200 deaths (Mead et al., 1996).
1999). Legitimate concerns over food borne illnesses have led to the formation of nationally recognized advocacy groups such as Safe Tables Our Priority, S.T.O.P. (Klinkhamer, 1998) and requests for improvements in national food safety strategies (DeWall, 1997). Fresh vegetables are probably only responsible for a very small fraction of these illnesses, but negative public attitudes towards the safety of fresh vegetables can be magnified by sensationalism in the media. In response, Cooperative extension has generated educational materials such as the pamphlet ‘Food Safety Begins on the Farm’ (Rangarajan et al., 1999). Yet, many growers are not convinced that they need to change their production practices to assure that produce does not come in contact with water that has been contaminated with microorganisms such as \( E. \text{ coli} \). In contrast, the general public has a growing concern over food safety issues related to microbial contamination (Powell, 1998).

Since reports of food-related illnesses are few, it is reasonable to assume that most food produced by Vermont growers is safe. However, there is a need for pro-active research to quantify the potential for microbial contamination and to identify the most likely sources of contamination in the Vermont vegetable industry. Such information will reduce the potential for contamination and improve efforts to educate the industry and the public about food safety. In addition, public awareness of increased food safety measures will lead to increased consumer confidence and loyalty and thus increased returns for Vermont vegetable growers. This proposal effectively addresses the VIRECA funding priorities of Vermont food safety and food security.

It is important to initiate this effort immediately for several reasons. First, preventing, rather than reacting to, food-related illness linked to Vermont’s vegetable farms is key to avoiding reduction in sales and economic loss. Second, many growers have no information about the quality of the water they currently use for vegetable irrigation and washing. Third, baseline levels of contaminants need to be established in order to assess future progress toward maintaining food safety as it relates to improved water quality. Finally, enhancing the safety of the public food supply is an important goal of agricultural research and extension in Vermont.

We propose to determine the point sources and extent of microbial contaminants on vegetable farms, evaluate sanitization protocols, educate growers on the findings, and educate consumers about their role in farm to table food safety. The development of extension resources will be necessary to educate and train the industry and public about the proper handling of vegetables from the farm, to food handlers, and on to the consumer’s table.

PREVIOUS WORK AND PRESENT OUTLOOK
There have been many endpoint studies on microbial contamination of fruits and vegetables. For example, food poisoning events in Korea and Japan were studied on a large scale between 1971 and 1990 and in both countries vegetables ranked third on the list of reported causes of illness (Lee et al., 1996). Furthermore, food borne illnesses have been traced to alfalfa sprouts, cantaloupes, watermelons, raw tomatoes, green onions, and raspberries (see review in introduction of Lopes, 1998). Salad ingredients purchased from supermarkets have been found to support coliform, yeast, and mold growth (Albrecht et al., 1995). Furthermore, storage at room temperature has been shown result in rapid increases in \( E. \text{ coli} \) O157:H7 levels on several fresh vegetables including shredded lettuce and sliced cucumbers (Abjul-Raouf et al., 1993). Abjul-Raouf (1993) also noted that outward appearance of the vegetables had no correlation with \( E. \text{ coli} \) O157:H7 growth. It was also shown that \( \text{Salmonella enterica} \) and \( E. \text{coli} \) O157:H7
will proliferate in oranges at 21° C following inoculation through a puncture wound (U.S. Food and Drug Administration, 1999).

Researchers have been working on methods to remove, kill, or prevent the proliferation of microbial contaminants. There have been several attempts to control *E. coli* growth and development with modified packaging atmospheres, but none of the modifications tested have been successful in reducing contamination (Abjul-Raouf et al., 1993; Hao and Brackett, 1993). Alternatively, linear furanocoumarins found in lime peel extract and cold pressed lime oil were found to be effective antimicrobial agents against *Lysteria*, however the treatments were ineffective against *E. coli* O157:H7 (Ulate-Rodriguez et al., 1997). Acid anionic sanitizers have been shown to not only kill 99.999% of bacteria in 30 seconds, but they have the additional advantage of not leaving residues on the surface of fruit and vegetables (Lopes, 1998). Simple chlorine washes have been shown to reduce microbial contamination, but not eliminate it (Albrecht et al., 1995). This mixture of positive and negative findings suggests there is still much work to be done in the area of food sanitation.

Scientists have also developed accurate and rapid tests to screen food for *E. coli* O157:H7 including colony hybridization (Samadpour et al., 1990) and PCR methods (Fratamico and Strobaugh, 1998). However, these identification techniques are currently used only for illness-outbreak investigations and research into food safety issues. These screening methods would be cost prohibitive for the small and diverse vegetable industry in Vermont. Therefore, Vermont efforts need to be concentrated on improving the understanding of microbial contamination potential and cost-effective prevention methods in the industry.

A CRIS search produced several projects that relate to food safety in vegetables. Johnson et al. (Project# ARK01781, Agency: CSRS ARK) are identifying factors that are involved in attachment and detachment of several common food-borne pathogens to raw produce to improve removal of these human pathogens during washing. Haddon et al. (Project# 5325-42000-021-00D, Agency: ARS 5325) has a similar project examining pathogen attachment to produce and poultry. Tamplin is also examining pathogen attachment to produce using several microbial contaminant delivery methods including artificial sewage (Project# FLA-FYC-03757, Agency: CSRS FLA).

Along a different but related research track, Nelson et al. are looking at pathogen inactivation by ozone and chlorine, among other gasses to improve the safety of fruits and vegetables for consumers (Project# IND 0600136, Agency: CSRS IND). In addition, Whitaker et al. are looking at controlled atmosphere packaging for not only fruit and vegetable quality preservation, but also human pathogen growth (Project# 1275-42430-001-00D, Agency: ARS 1275). Sumner (Project# VA 135563, Agency: CSRS VA) is looking at processing and packaging techniques to reduce microbial contamination of fresh fruits, vegetables, and juice including washing with acetic acid and an acetic acid H$_2$O$_2$ combination.

Our proposed research examines not only the preparation of fresh vegetable produce for retail markets with different sanitization treatments to reduce microbial contamination, but surveys farms for microbial contamination potential. This research if funded will allow growers to be presented with a realistic risk assessment of microbial contamination in addition to sanitization recommendations. We will also address the needs of conventional and organic growers by examining disinfecting treatments that are compatible with both farming systems.
Health and Human Services Secretary Shalala stated in a recent address that “nearly a quarter of all Americans are considered “at risk” from exposure to food borne pathogens” (Department of Human Health and Services, 1999). A 1996 survey found that bacteria/germs were the number one food attribute concern for consumers (FMI data as presented by (Powell, 1998)). However, the actual risk of food borne illness originating on the farm isn’t well scientifically documented, although great strides have been made in isolating organisms responsible for outbreaks and developing anti-microbial post-harvest treatments and protocols. The proposed research and extension activities will improve the understanding of microbial contamination potential on Vermont vegetable farms, provide sanitation recommendations to maximize food safety, and fill the “information vacuum” that so often exists between the scientific and public perceptions of risk (Powell and Leiss, 1997). Ultimately, these deliverables will lead to lower risk (both real and perceived) and increased food safety confidence for Vermont vegetable consumers and reduced liability for growers.

OBJECTIVES

1. Determine the point sources, distribution, and frequency of occurrence for specific microbial contaminants by sampling irrigation water and wash water from vegetable farms in Vermont. (years 1 and 2)

2. Evaluate protocols recommended to minimize microbial contaminants on produce and determine which protocols can be most effectively and economically transferred to vegetable farms. (year 3)

3. Educate growers on the findings of the project and consumers about their role in farm to table food safety through a series of journal articles, newsletter articles, fact sheets, presentations, Internet publications, and workshops. (years 1, 2, and 3)

PROCEDURES (organized by objective)

1. *Determine the point sources, distribution, and frequency of occurrence for specific microbial contaminants by sampling irrigation water and wash water from vegetable farms in Vermont. (years 1 and 2)*

   Based on suggested measures in “Guide to Minimize Microbial Food Safety Hazards for Fresh Fruit and Vegetables” (U.S. Department of Health and Human Services et al., 1998) water samples will be taken from organic, sustainable, and traditional farms. Identities of industry participants in the project will be kept confidential. Sample sites at each participating farm will include:

   1) irrigation water source
   2) irrigation water at the emitter or drip line
   3) vegetable wash water pre-wash
   4) vegetable wash water post-wash
   5) rinse water from produce on the shelves at farm retail outlets.

   **Sample collection schedule:**
Six participating vegetable farms will be surveyed for microbial contamination throughout the growing season for two consecutive years. Samples will be taken during the growing season to examine weather and seasonal change effects (heavy rains can result run-off events from a variety of sources) on potential microbial contamination. Based on findings, a model farm will be chosen to implement 6 different sanitization procedures. Alternatively, in year 3 we may conduct the sanitization treatments under controlled laboratory conditions if environmental variables are determined to be too difficult to control on the farm. For instance, in the laboratory it would be possible to inoculate produce samples with a known amount of harmless bacteria and then test water at various stages under different treatments for microbe levels. Treatments will include chlorinated wash water, peroxide treated water, commercial agents such as Vircon, acid anionic sanitizers, and distilled water washes regardless of whether a farm or lab is chosen as the test site for year 3. Sample collection will take place throughout the growing season. Sample analysis will be contracted out to a Vermont Department of Health approved water quality testing laboratory. Quantitative analysis of total coliform and fecal coliform organisms will be conducted using the membrane filtration method. Two labs have been identified that will conduct these tests for approximately $30.00 per sample (Analytical Services, Inc. Williston, VT and Sci Test, Inc. Randolph, VT).

Total coliform organisms are common in many soils and positive test results are not an absolute indicator of a contamination issue. However, with high coliform numbers it is suggested that an investigation take place to ensure there are no hidden greater risks to the water supply (New Hampshire Department of Environmental Services, ). Fecal coliforms are a sub-group of the total coliform group and usually originate in mammal intestinal tracks. There is a much higher risk of improper disposal of waste materials when fecal coliform numbers are high. It is usually mandated that private and public users are notified of a potential problem due to the higher probability of risk as compared to high total coliform counts (New Hampshire Department of Environmental Services, ). E. coli, a species of coliform bacteria, are only associated with the intestinal tracks of animals and are a stronger indicator of sanitary waste in the water supply than either total or fecal coliform counts (New Hampshire Department of Environmental Services, ). However, due to the cost of this analysis we will concentrate only on total coliform and fecal coliform numbers, which will allow us to cover many more farms and water sources. If we find substantial evidence of a pattern of contamination we will seek additional outside funding sources to further define levels of specific pathogenic organisms.
organisms.

Leafy greens have been chosen as the model crop for the research based on the following reasons. Fresh leafy greens have been growing in popularity for the last decade. Fresh greens are also often washed on-site to remove field soil, dust, and other debris. This crop also has the possibility of being shipped and stored in a high-humidity (bagged) environment. These conditions facilitate microbial growth and increase the chances of a contamination event. There may also be some consumer confusion over the sanitary level of washed leafy-green materials due to packaging and presentation of other fresh produce products. Some supermarket packaging labels include statements to the effect of “washed,” “triple-washed,” and even “washed and ready to eat.” Most small growers wash only to remove debris not to prevent microbial contamination. Washing and packaging of fresh leafy greens do not eliminate the need for thorough cleaning prior to consumption.

2. Evaluate protocols recommended to minimize microbial contaminants on produce and determine which protocols can be most effectively and economically transferred to vegetable farms.

During the third year of the study recommended procedures such as rinse water chlorination (Grubinger, 1999) and other alternative techniques (see review by Majchrowicz, 1999)) will be compared to traditional washing techniques at one farm or in the laboratory. The treatments will be targeted to problem areas determined during the first year of the study. If no problem area trends are identified then treatments will be designed based on HACCP principles from other crops (see review by Rushing and Ward, 1999). Treatments will be designed with sustainable and organic growers in mind. For example, recommended strengths of chlorinated wash water to reduce contamination by pathogens exceed the limits allowed in organic farm certification regulations (Grubinger, 1999). The proposed Federal Organic Rule allows for chlorine in vegetable wash water at concentrations not to exceed the Federal drinking water standard (4 ppm). That level is unlikely to assure food safety if produce is contaminated, so there is a strong need for alternative sanitizers to be identified for use on organic farms.

3. Educate growers on the findings of the project and consumers about their role in farm to table food safety through a series of journal articles, newsletter articles, fact sheets, presentations, Internet publications, and workshops. (years 1,2, and 3)

Outreach methods will include: articles in the biweekly Vermont vegetable and berry growers’ newsletter (electronic and in hardcopy in the VT Department of Agriculture’s ‘Agriview’), fact sheets to be mailed to all producers and distributed upon request. In addition, there will be several on-farm workshops or ‘twilight meetings demonstrating sampling techniques and washing systems that are effective. Investigators will make presentations at the Vermont vegetable and berry grower’s association during the winter meetings in Barre and Rutland, and at the New England Vegetable and Berry conference in Sturbridge, MA in Dec. 2001. There will also be individual consultations with growers covering procedures to reduce the probability of microbial contamination of fresh vegetables targeted at the retail market.
Additionally, talking points and point-of-purchase information to be used by any and all growers will be developed to distribute to the public and respond to the media when the situation arises. These information resources will be developed with grower input and reviewed by UVM Extension personnel with expertise in Food Safety.

**DURATION**

This project requires 3 years to complete.

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<td>1</td>
<td>October 1\textsuperscript{st}, 2000 – September 30\textsuperscript{th}, 2001</td>
<td>Farm sampling and testing, presentations to growers on project goals, assembling known protocols for fresh vegetable sanitation</td>
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<td>2</td>
<td>October 1\textsuperscript{st}, 2001 – September 30\textsuperscript{th}, 2002</td>
<td>Farm sampling and testing year two, presentation to growers of year 1 results, determine test treatments for third year</td>
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<td>October 1\textsuperscript{st}, 2002 – September 30\textsuperscript{th}, 2003</td>
<td>Testing of sanitization improvement recommendations, presentation to growers of year 2 results, publication of findings year 3 data analysis, publication of grower fact sheets with recommendations based on findings, development of fact sheets targeted at fresh vegetable consumers.</td>
</tr>
</tbody>
</table>
LITERATURE CITED


Grubinger, V. (1999). “Post-harvest washing of fresh produce to reduce food safety risks.”, University of Vermont Extension


New Hampshire Department of Environmental Services. “Interpreting the presence of Coliform bacteria.” WD-WS-4-1, Concord, NH


