Title: Agricultural water quality and transfer of foodborne pathogens in grapes and strawberries.

Progress Report: grant is finished in 2008 but research will be finished in spring 2009.

SRSFC Project # 2008-15

Research Proposal

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Objectives
Develop an understanding of the microbial quality of water used for growing grapes and berries and how the microbes in this water transfer to the fruit

Justification
With a number of recent outbreaks, the safety of fruits and vegetables is becoming a major priority for both growers and consumers. In response to these outbreaks, packers and retailers are starting to require growers to follow good agricultural practices and various mandates are being presented by commodity groups. For example, the California leafy greens marketing agreement is being used as an industry standard in the production of leafy greens and in other crops (such as Florida's tomato GAPs program). These mandates introduce standards of practice for employee hygiene, handling of animal manure for fertilizer, field sanitation, packing house operations, transportation, product traceback and of course, water quality and use. While the GAP recommendations were first published as guidance by the FDA in 1997, many of the recommendations are merely best estimates from various academics and while useful, much work is still needed to confirm these recommendations. Water, for example, is required to be tested three times during the season. However, when a grower gets his water test results back from the laboratory and asks the question, "What does an MPN of 85 actually mean?," they will often get an answer from the laboratory or the horticultural extension agent describing the MPN procedure and a statement about only using potable water if it is going to come in to contact with the edible portion of the plant. They receive little information about how to maintain or improve water quality on their farm, what to monitor between the three sampling dates to know when it may not be not safe to use surface water, or what their options are for treating water that test results indicate is outside of recommendations. Further, the standards being instituted were developed in California using recreational water as a standard. While these may still be appropriate, the water quality in the southeastern United States may be quite different. Specifically, there has been no investigation of the quality of the surface water often used to irrigate produce in the Southeast. Also, little is known about the transfer of microbes by agricultural water used in various production practices. Recreational water standards may be too stringent but recreational water provides the only current science-based guidelines.

Methodology
In this project, we will begin to study this issue in southeastern North Carolina. First, we will sample the surface ponds at the Horticultural Crops Research Station in Castle Hayne, NC throughout the year. The methods we proposed in the project were as follows; Three 500 ml samples will be taken from different parts of the pond using sterile containers for each sampling. Each month, samples will be removed from the pond at the same three spots. One hundred milliliters will be tested using the Colilert test to provide standard coliform counts. We will also test for *Salmonella*, *Escherichia coli* O157:H7, *Cyclospora* and *Cryptosporidium* using the methods outlined in the FDA's Bacterial Analytical Manual. This will allow us to develop a baseline for the water quality at the

research station. A portion of the sample will be frozen at -80^{o}C for future testing using more sensitive molecular biology based methods.

Water samples from muscadine grape and strawberry production areas will also be tested prior to irrigation, before mixing and spraying of pesticides and before the use of water in frost protection or evaporative cooling. After the fruits' exposure to water through pesticide application, frost protection or irrigation, samples of the fruit will be tested for coliforms, *E. coli* O157:H7, *Salmonella, Cyclospora* and *Cryptosporidium*. Fruit samples will then be collected 1, 3, 7 and 10 days later to determine if any microbes transferred from the water will persist on the fruit. These samples will be compared to a control sample taken prior to the agricultural water exposure using appropriate statistical methods to determine if the treatment is responsible for an increase in the microbial population on the fruit. It is hoped that this project will provide preliminary data for a larger collaboration to test agricultural water throughout the southeastern United States and determine its role in the transfer of pathogens to various crops.

While over 40 samples where collected from both Castle Hayne Research Station and Maxwell Creek Vineyards to track the microbial level of the agricultural water and the transfer of these microbes to microbes to the fruit. We discovered issues with the samples and travel to the University of analysis during this time some of the samples where stored improperly and the viable microbes destroyed before analysis. Thus we can no longer use the proposed methods to analyze the grape samples. Therefore we will now use a much more sensitive molecular method (real-time PCR) to detect and quantify the coliforms, E. coli O:157:H7 and the parasites.

In regards to the transfer of microbes to strawberries during frost protection, we have changed our research method. As other project in our laboratory have demonstrated a large amount of variation in generic E. coli counts that would make interpreting data on transfer of microbes to strawberries difficult we have decided to spike the irrigation water used for frost protection with a surrogate, coliphage, which is used to mimc viruses and is nonpathogenic, in fact it only affects bacteria. Using this we will be able to determine the transfer of the surrogate to the strawberries during frost protection and as described above we will determine its persistence on the plant itself. This portion of the project will start during February and March of this year.

Results

Due to the issues outlined above, improper storage of the grape samples and the project starting after the frost danger for strawberries we do not currently have results to report but anticipate these being available in the spring.

Conclusions

None to date

Impact Statement
We anticipate developing a better understanding of the role of water in the transfer of pathogens to produce. Additionally we will be able to provide some guidance to strawberry growers about the impact of overhead irrigation on frost protection.

Citation(s) for any publications arising from the project
None to date