CPS Technical Symposium Key Learnings

June 23, 2010

1. **Pathogens do not survive well in the production environment**. Attenuated *E. coli* O157:H7 applied by a spray so it simulates what might happen if an overhead irrigation water source were contaminated with the pathogen, does not survive well on the surface of either spinach or lettuce. The pathogen dies off quickly so that it is very hard to detect after 2 days.

2. **Pathogens are not taken up through the roots of a plant**. Attenuated *E. coli* O157:H7 delivered to the roots of growing spinach plants via drip irrigation does not traverse the root and get taken up by the plant under agricultural production conditions.

3. **Pathogens do not seem to move through the soil**. Attenuated *E. coli* O157:H7 inoculated into the soil or sprayed on top of the soil did not survive past seven days and was not shown to move from the inoculation site.

4. **Pathogens may survive for longer periods when associated with organic matter**. Spinach inoculated with *E. coli* O157:H7 and turned under the ground was recovered out to 100 days. The cultivation practices used were not typical of current production practices so this work will be repeated in 2010 using production practices for preparing fields to replant.

5. **A technology to permit storage of pathogen DNA can aid investigations**. FTE filter papers can be used to store DNA from bacteria for up to ten months at room temperature. Often the limiting step in taking samples to investigate a potential contamination event is the number of samples that can be processed and analyzed. By storing sample extracts on filter papers, virtually hundreds of samples can be taken and then assayed at a future time as analytical capacity becomes available.

6. **A "perfect storm" can result in pathogen growth**. Moisture, temperature and perhaps other environmental factors can create conditions where pathogens, if present, can survive and multiply. Rapid response experiments show that rainfall followed by warm temperatures created an event where pathogens were detected in both raw and finished products. Multiple genetic variants of the *E. coli* O157:H7 were also recovered.
7. **LGMA "buffers" appear to work**. Rapid response experiments show that following a field intrusion by feral pigs, elevated levels generic *E. coli* was found where the pigs obviously contacted the crop, but not out beyond the 10 foot buffer zone.

8. **Larger sample sizes increase the chance of finding pathogens**. Typical commercial product sampling procedures use 25-gram samples to test for pathogens. Data was presented that shows increasing the sample size to 150-grams increases the chance of detecting low level contaminations.

9. **Non-pathogenic bacteria may be used to identify conditions that permit pathogen survival**. Preliminary characterization of natural bacterial populations that exist on the surface of leaf vegetables change by location and season. Some bacteria may be used to indicate the conditions are supportive of pathogen survival while others have been shown to be antagonists of pathogen survival. In a risk-based testing system, these non-pathogen bacteria could be used in conjunction with other measurements as "indicators" to identify when there may be an elevated risk of pathogen contamination or survival.

10. **Filth flies may be a potential vector for E. coli O157:H7.** Flies have the capacity to transmit *E. coli* O157:H7 to the surface of vegetables under laboratory conditions. A very low prevalence of flies captured near vegetable production fields test positive for *E. coli* O157:H7. It is not known if this risk factor is significant and more testing needs to be done.

11. **Simple modifications to lettuce coring knives can significantly reduce the risk of pathogen contamination.** Though reliant on the unlikely event of a coring knife coming into contact with an un-naturally high concentration of pathogens in the field, transfer of pathogens to cut surfaces has been demonstrated previously. By extending the common coring tool away from the cutting blade, the chance of cross contamination is greatly reduced. Further, by polishing joint welds, the tools are much easier to sanitize thereby further reducing cross contamination frequencies.

12. **Modifications to existing molecular methods may permit rapid, inexpensive and more sensitive assays for *Salmonella*.** LAMP technology has been shown to have the potential to provide quantitative assay of Salmonella that is ten times more sensitive than PCR. The use of an intercalating agent permits the assay to distinguish between live and dead cells. Additional experiments are required to demonstrate selectivity in complex produce chemical environments.

13. **Preliminary data suggest that sheep can be carriers of *Salmonella***. Bands of sheep sampled for *Salmonella* were shown to be carriers. In these experiments, no bands were found to carry *E. coli* O157:H7. Further experimentation is required to determine if sheep grazing in vegetable production environments actually represents a significant contamination risk.

14. **Improper composting can result in pathogen survival**. Moisture, "heat-up times", temperature, turns and other factors significantly affect the ability of pathogens in compost to survive the process. Further, if a validated process is not followed, pathogens develop heat tolerances and obtain a higher level of survivability.

15. **Bacteriophages may be a useful tool to improve the sensitivity of assaying finished compost for pathogens**. It has been difficult to develop reliable tests for pathogens in complex organic backgrounds like compost where many non-pathogenic species are also present. Using bacteriophages to kill competing species can improve pathogen recovery and PCR sensitivity.