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Field Trials Using Surrogate Vials Help Debunk 90/120-day Manure Rule

Much like the hosts of the Myth Busters TV show who try to prove or disprove myths, Keith Warriner and his team are using science to verify or debunk the 90/120-day manure rule. Also known as the "manure waiting period" - the rule permits use of soil-incorporated raw manure a minimum of 90 days before harvest for crops where the edible portion won't contact it. It also allows the use of soil-incorporated raw manure at least 120 days preharvest for crops, such as carrots and potatoes, where the edible portion will contact it.

"It's all about giving the farmers tools for risk management," said Ann Huber, Ph.D., project co-investigator and an environmental microbiologist with the Soil Resource Group in Guelph. "Whatever we do, it has to fit into their production system." With the project now complete, the data is being used to develop algorithms that eventually may be incorporated into a smart phone application for growers, Warriner said. Using local weather data, soil type and preharvest intervals entered by the grower, the app would provide the relative pathogen contamination risks associated with using raw manure.For the past two years, Warriner, Ph.D., and a professor in the University of Guelph's Department of Food Science, Ontario, helped lead a group of researchers, industry representatives and Ministry of Agriculture officials on the project designed to bring science into the manure risk-management equation.

"Manure, if applied correctly, can be a valuable source of nutrients and organic matter, Warriner said. Just by its nature, manure may contain pathogens detrimental to human health. In many cases, growers avoid using manure altogether to reduce any associated risk. Other researchers have tried to quantify manure pathogen die-off rates in laboratory trials. But as Warriner pointed out, "Obviously, it's under controlled conditions. That's a big problem because it's not representative of the soil, rain and temperature fluctuations that occur during the day in the field."

One of the challenges with conducting this type of research under natural conditions is scientists have been reluctant to introduce pathogen laced raw manure into the environment. Even if they found a willing cooperator, it would be nearly impossible to separate pathogen population reductions caused by die-off from organisms that were simply washed away.

To address those research challenges, Warriner and his group used small sentinel vials filled with a manure and soil mixture. The vials contained the pathogens so they're retained and not washed away but still allow the organisms to be subjected to environmental conditions within the soil. The researchers used two different manures - liquid dairy and liquid swine - because of differing constituents within each waste produce that could affect pathogen viability. Each vial was inoculated with a known population of: *Salmonella* or Shiga toxin-producing *E. coli.* *Clostridium difficile* was included as an emerging pathogen of interest to public health officials.

The researchers placed one set of vials in a 15-centimeter-deep trench covered with soil to simulate manure injection, which has been promoted as a way to manage manure nutrient run-off. Another set of vials was placed on top of the soil to reflect surface manure applications. They also conducted the trials during the spring (May) and fall (October) as well as in two different soil types - loam and sandy loam. An automated weather station recorded data, such as precipitation, soil temperature and air temperature. During the trial, the researchers monitored pathogen die-off rates and how long it took populations to be reduced two logs.

Warriner was quick to point out that the research was conducted in only two soil types and only in Ontario. "How do we know that the die-off will be the same in California, in Arizona or in Florida? Not to mention the role of endogenous microbial populations on pathogen survival. That's why we need to collect more data, because the more data you have, the better models you can develop." He said he envisioned the system being used by other researchers as a foundation "to get standardized methods so people all use the same techniques." Regardless of the manure, organism, soil type of depth, none of the treatments required even 90 days to achieve a two-log die-off although low residual sub-populations of pathogens persisted beyond this time. In general, pathogens in vials placed on the surface had a faster die-off rate than those in the paired treatment buried in the trench. Pathogens in vials on the sandy loam soil surface had the fastest die-off, ranging from less than four days to 10 days. That compares to vessels buried in sandy loam, where die-off ranged from four to 28 days. On the other hand, pathogen in vials on the loam soil surface had die-off rates ranging from less than four days to 26 days compared with the buried vials, where die-off rates ranged from four to 64 days.

Key Industry Take-Aways

• The die-off rates of pathogens may be predicted taking into account soil and manure type, climate and surface/subsurface application.

• The research can ultimately lead to onfarm risk management tools such as apps that can be used to predict safe preharvest wait times.
• Non-pathogenic E. coli is a conservative surrogate for pathogen risk in most instances.

View the final project report on the CPS website: Die-off rates of human pathogens in manure amended soil under natural climatic conditions using novel sentinel chamber system

CPS Contact:
Bonnie Fernandez-Fenaroli, Executive Director
Phone (530) 554-9706
Email bonnie@centerforproducesafety.org