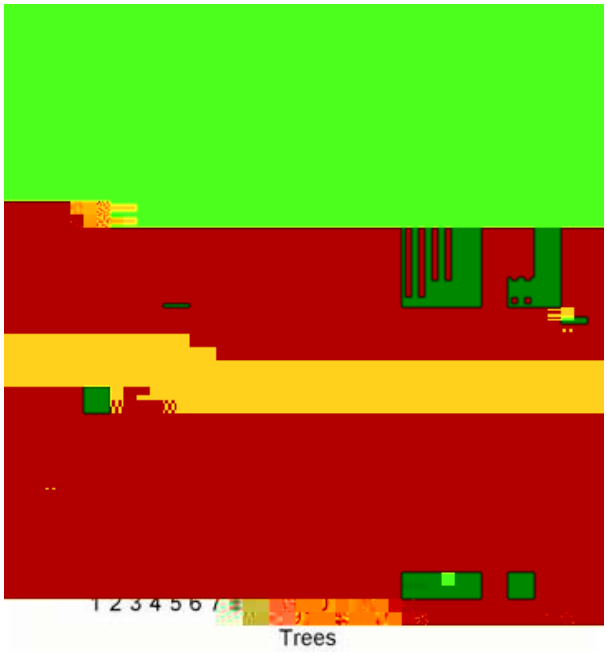
In a previous article (http://primera.tamu.edu/kcchome/newsltr/december2003.htm) dated December 2003, we reported an improved Baermann funnel technique for citrus nematode assay. This improved assay utilizes a membrane filter. Membrane filter (MF) technique is a standard procedure used worldwide to assess bacterial contamination in water. This technique is simple and has use in several different applications in addition to bacteriology. The technique allows filtration of a large volume of water to retain microorganisms and particulates on a membrane placed on a filter holder with a seal to prevent water leak. We have adapted the bacterial MF technique to screen a large volumes of soil samples to assay for the citrus nematode, *Tylenchulus semipenetrans*. The stored membranes can be kept in a refrigerator for months and can be re-evaluated for counting, photography, and/or scanning.

: We used 0.45 µm pore size, circular (47 mm diameter) cellulose nitrate filters from Whatman Inc. (Clifton, NJ) and Gelman Sciences Inc. (Ann Arbor, MI). Cellulose nitrate filters are used in general purpose analytical filtration, when protein binding is not important. It has good wetting property and has a fast flow rate with aqueous solutions. The membrane has a total of 151 full squares (each 9 mm^2 size). The filters are placed on a filter holder and are available either sterile or non-sterile.

: The Baermann funnel procedure is a routine process for extracting drawing-out nematodes from soil and root. In its simplest form, soil samples (with or without root pieces) are placed on tissue paper on a wire-screen support. The wire screen is placed on top of a funnel filled with water. The funnel tip is extended with rubber tubing that is sealed with a clamp. Additional water is added to keep the soil moist. Juvenile nematodes leave the soil and roots, pass through the wire-screen support and accumulate at the end of the funnel.

C : Adult females of citrus nematode, *Tylenchulus semipenetrans* feed on feeder roots. The posterior part of the body is outside the root, extruding a gelatinous material into which eggs are deposited. The life cycle from an egg to the next generation eggs requires 4 to 8 weeks. Second-stage nematodes emerge from eggs. Males are smaller and do not feed. The second-stage female undergo four molts or form-changes. They penetrate and embedded in the feeder roots. The citrus nematode also reproduces on olives and grapes.

: In March 2005, we evaluated more than 300 soil samples using the MF technique. This study was part of our research on citrus nematode management in orchards using new products, viz., a biological control agent, a growth regulator, and an insecticide. The studies were conducted under flood, drip, or micro-sprinkler irrigation. We found the MF technique accurate, simple, and it provides us with a long-term record. In drip irrigated orchards, the nematode population around the drip emitters was found to be consistently higher than the population at the drip line of the tree. The following chart shows results from 10 trees. The bars on the right represent the population of citrus nematodes at the emitters and the bars at the left show sampling results from the drip line away from the emitters.



: One of the major limiting factors in assessing nematode populations in field soils is the cost of laboratory analysis. Moreover, high sample numbers are required to obtain realistic information of nematode infestation levels in a large field. The extraction

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