An Improved Technique for Clearing and Staining Plant Tissues for Detection of Nematodes

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Numerous procedures have been used to stain and clear nematode-infected plant tissues (7). Staining with acid fuchsin and destaining with lactophenol (4,7) has been the most widely used technique. More rapid means of clearing and staining roots include autoclaving (1), exposure to saturated chloral hydrate (6), and root maceration after staining (3). However, extreme care is required for these methods.

Researchers working with mycorrhizal fungi found potassium hydroxide (KOH), nitric acid, hydrogen peroxide (H_2O_2), or sodium hypochlorite (NaOCl) to be effective in clearing roots (2,5), depending on the type of tissue to be cleared. Alkaline H_2O_2 was the best bleaching agent for pigmented roots (5). Excessive bleaching with NaOCl often resulted in poor staining of tissues.

A modified acid-fuchsin staining-destaining procedure utilizing NaOCl as a prestaining treatment for nematode-infected plant tissues is described herein.

Roots are washed, chopped into 1–2-cm segments, and placed in a 150-ml beaker with 50 ml tap water. Twenty milliliters of chlorine bleach (5.25% NaOCl) are added to give 1.5% NaOCl, and the root tissue pieces are allowed to remain in this solution for 4 min with occasional agitation. Following the NaOCl treatment, the root segments are rinsed in running water (30–45 sec) and allowed to soak in tap water for 15 min to remove residual NaOCl. The material is then drained and transferred to a beaker containing 30 ml of water to which has been added 1 ml of stain (3.5 g acid fuchsin, 250 ml acetic acid, and 750 ml distilled water). This solution is then heated to boiling for about 30 sec.

After cooling to room temperature, excess stain is removed by rinsing in running water. The root material is then placed in 20–30 ml of glycerin acidified with a few drops of 5N HCl, heated to boiling, and cooled. The root segments can then be pressed between glass plates or microscope slides for observation.

The NaOCl-acid fuchsin-glycerin technique has given good results with soybean roots infected with *Heterodera glycines* Ichinohe (Fig. 1–A) and cotton roots infected with *Meloidogyne incognita* Kofoid & White (Chitwood) (Fig. 1-B). This technique results in high quality specimens in young, succulent root tissue. In older or more ligneous roots such as cotton or corn, some difficulty in clearing or destaining may be encountered. Two methods have given satisfactory results with older cotton roots infected with *M. incognita:* i) chlorine bleach pretreatment can be increased to 30 ml per 50 ml of tap water (2.1% NaOCl), or the pretreatment exposure time may be increased to 5–6 min; and ii) roots may be soaked in 30% H_2O_2 for approximately 1 h, rinsed, and stained.

Phillips and Hayman (5) found that cotton roots heated in 10% KOH prior to treatment with alkaline H_2O_2 (10 min–1 h) allowed rapid and relatively easy detection of fungal infections. However, we found that extreme care must be taken in the use of KOH in nematode-infected roots or the nematodes may be destroyed. Adequate root clearing was achieved with a 1-h exposure to reagent grade H_2O_2 (30%).

Use of either clearing agent (H_2O_2 or NaOCl), when combined with glycerin for destaining, eliminates exposure to toxic phenols. This method also minimizes the time required for specimen preparation. When acidified glycerin is used, destained root material can be stored for several months with very little change in the initial contrast between nematodes and root tissue.

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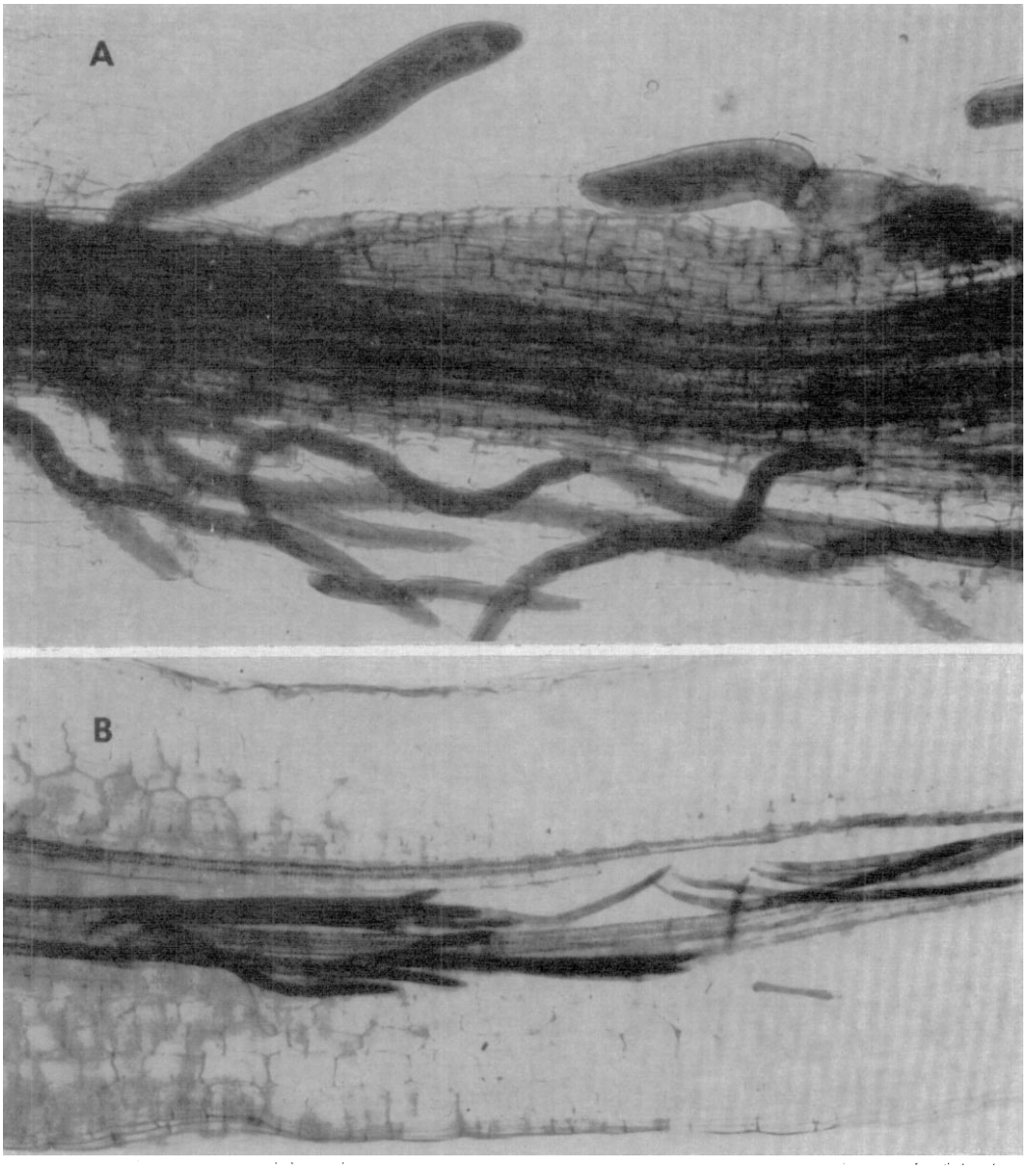

Fig. 1. Nematode-infected plant roots stained with NaOCl-acid fuchsin-glycerin technique. A) Soybean roots infected with *Heterodera glycines*. B) Cotton roots infected with *Meloidogyne incognita*.

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