

# Detection of fungal infections in plants



## Histopathological Technique for Detection of Fungal Infections in Plants

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## Abstract

Microscopic examination of the interaction between pathogenic fungi and their host plants has been instrumental in deciphering the biology of this relationship and can serve as a useful diagnostic tool. In this chapter, we describe the technique of fixing fungal infections of plant samplings for histopathological experiments. Toluidine blue O' staining methods coupled with stereoscopic microscopy are used to scan the infection structures of the fungus *Fusarium* spp. and host response in *Psidium guajava* L. root tissues.

Key Words: fungal infections, histopathological experiments, microscopy, staining techniques, Toluidine blue O', *Fusarium* spp., *Psidium guajava* L.

**INTRODUCTION**

The ability to observe the growth of fungal structures in host tissues under the microscope is an important tool in the study of plant pathogenesis. Over the years many staining techniques that highlight fungal structures in plant tissues have been reported. In particular, technologies such as stereoscopic microscopy have enhanced our ability to visualize hyphae in plant tissue. [1-4]

The use of certain staining techniques can facilitate considerably microscopic observations and experimental research on plant pathology by allowing plant and fungal tissues to be differentiated. More specifically, staining can aid examination of fungal colonization and infection processes, such as

differentiating hyphae in life cycles that involve a transition from a biotrophic to a necrotrophic phase. Staining of specific tissues also can simplify identification of fungal inoculum or hyphal presence in asymptomatic plant tissue. The effectiveness of a particular staining technique can vary greatly depending on the particular fungus and plant species. Toluidine blue O' has been used to stain and identify callose deposition produced by host plants in response to intracellular infection of plant cells by fungi in some plant-fungus interactions.[5] Toluidine blue O' staining techniques was applied to examine the infection structures of the fungus *Fusarium* in *root* tissues wilt infected guava plants. The usefulness of this staining method was based on the visual contrast between host plant tissue and fungal hyphae provided by polychromatic dye and resolution, and the relative ease of preparation and use. [6] This study describes an improved method for fixation of sampling of fungal infected plant parts, and staining and observation of fungal infections in plant tissue for histopathological visualization.

## **MATERIALS**

1. Sterilized water
2. 0. 1% HgCl<sub>2</sub>
3. Glass slides
4. Whatman filter paper no. 41
5. Formaldehyde
6. Glacial acetic acid
7. Alcohol
8. Xylene
9. Paraffin wax

10. Toluidine blue O'
11. DPX-mount
12. Microprocessor based automatic tissue processor (Electra, YSI 104, Yorko )
13. Microtome (MICROM - HM 350)
14. Stereoscopic microscope (Leica - LEITZ - DM RBE)

## METHODS

The methods presented in the following sections describe general procedures for fixation, staining, and microscopy of fungal infections of plant samplings. Modifications that may be needed to fix the sampling properly from different types and sources of material are also described.

### Killing and Fixation

Roots samples were collected from wilt-affected and healthy plants. Root pieces 2 to 4 cm long were cut and surface sterilized using 0.1% HgCl<sub>2</sub>, washed two to three times in sterilized water, and the excess water absorbed on Whatman filter paper 41. Then samples were kept in formaldehyde: acetic acid: alcohol (5 ml: 5 ml: 90 ml) for a minimum of 48 hours (see Note 1).

### Dehydration

The samples were processed with the alcohol: xylene series (as per the flow chart depicted in Figure 13.1) using an automatic tissue processor (Yorko) (see Note 2).

### Infiltration and Embedding

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The samples were embedded in melted paraffin wax (54-56° C) for at least 4 to 8 hours in order to completely replace the xylene with paraffin wax in a square-shaped block (see Note 3).

### Sectioning

Section (10  $\mu$ m thick) cutting was done using a microtome. Blocks were prepared in paraffin wax and thin sections 10  $\mu$ m thick were cut with the help of a microtome (MICROM - HM 350S). At least 20 slides were prepared for each sample (see Note 4).

### Staining and Mounting

The sections were stained in 0.1% aqueous toluidine blue O' and were mounted in DPX after bringing them to xylene through the alcohol: xylene series. The detailed procedure is given in the flow diagram depicted in Figure 13.2 (see Note 5).

### Microscopy and Imaging

Samples were mounted in 50% (v/v) DPX mount and viewed under a stereoscopic microscope (Leica - LEITZ DM RBE) using a Hoya CM500S filter (IR cut-off 650 nm). Images were captured using a CCD camera with a Bayer Array RGB filter for brilliant pictures (Interline transfer frame readout CCD - ICX252AQ) and Leica DFC Twain and Leica Image Manager analysis software (soft microscopy with imaging control software system).

## NOTES

1. The FAA solution is prepared based on the type of material, that is, soft tissue, moderate tissue, or hard tissue (use 25% ethanol for very delicate material, 50% for normal use, and 70% ethanol for very tough material). The samples were left in the FAA solution at least 48 hours or until they were processed further. This depends on the hardness of the tissue.
2. This process removes the water from the plant tissues and facilitates sectioning.
3. Infiltration and embedding of the material was done in paraffin wax to remove the xylene from the tissues. The blocks of wax were prepared in L molds in which the material was embedded.
4. Sectioning of the material was done with the automatic microtome (MICROM – HM 350 S).
5. Sections were stained in 0. 1% aqueous Toluidine blue O' and were mounted in DPX after bringing them to xylene through the alcohol: xylene series as described by Jensen. [7] The samples were examined for anatomical details as per the technique described by Pandey. [3]

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