

# STAINING FOR PERCENTAGE AM COLONIZATION

([INVAM](#), Giovannetti and Mosse, 1980)

## Materials

250 or 500 um sieve

10% KOH

1% HCl

bunsen burner or hot plate

beakers

tissue cassettes, vials or test tubes

weigh boats

paper bags

Petri plates (with grid, optional)

Grid paper with grids of a known size

0.05% Trypan Blue stain [33 ml lactic acid, 33 ml glycerol, 33 ml water, 0.5g Trypan or Direct Blue]

## Methods

- 1) Wash roots with forced water over sieve to remove soil.
- 2) Place root sample in tarred weight boat and record total weight.
- 3) Weigh paper bag (either open in a standing position or in tri-fold position – just make sure that all of bag is on the weighing pan). Record weight on bag and on data sheet.
- 4) Remove a root subsample (>0.2 g, preferably at least 0.5 g) and place in paper bag. Record weight. This will be used to determine total dry weight. Dry at 70 to 90°C. Weigh after completely dry and calculate the percentage of water in the subsample and use this to calculate the total dry weight of the root sample (see example below).
- 5) Take remaining roots or another subsample of roots and place in tissue cassettes, vials or test tubes for staining.
- 6) Roots may be stored at 4°C like this for a week or so before staining. (If they need to be stored for a longer period, place the roots in a 1:1:1 lactic acid: glycerol: water solution at 4°C.)
- 7) Bring 10% KOH\* solution to a boil\*\* and pour over roots. Incubate for 5 min.
- 8) Remove KOH and rinse with tap water 5 times.\*
- 9) Add RT 1% HCl and incubate for 5 min.
- 10) Meanwhile, bring 0.05% Trypan Blue stain to boil.\*\*
- 11) Remove HCl solution\* and add stain. Incubate 5 min.
- 12) Remove stain and store in water at 4°C. (Note: Stain is a hazardous waste, dispose of properly.)
- 13) If a long term storage is desired, store in 1:1:1 acid:glycerol:water solution at 4°C in air tight containers. Monitor for fungal contamination and if roots become to destained, repeat steps 7-10.
- 14) Determine observe stained roots (Fig. 1) and determine percentage colonization via grid-line intersect method outlined in Giovannetti and Mosse, 1980.

\* KOH solution, HCl solution, and rinses from KOH may be combined for neutralization prior to disposal.

\*\* Alternate method:

1. Leave KOH at room temperature and incubate roots at least overnight.
2. Leave Trypan Blue stain at room temperature and incubate overnight.

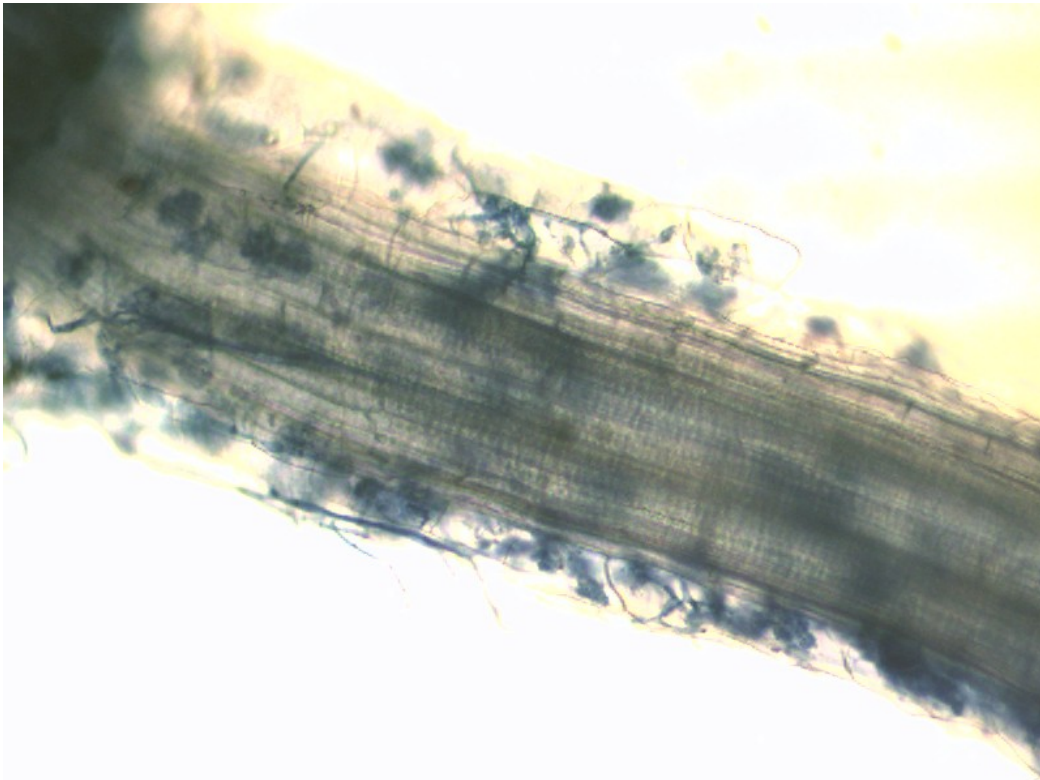
**Example of equations used to determine the total dry weight of roots:**

Wet weight of entire root sample = 5.00 g

Wet weight of subsample = 0.50 g

Dry weight of subsample = 0.05 g

$$\begin{aligned} \text{Total dry weight of roots} &= (\text{Wet weight of entire root sample}) * \\ &\quad (\text{Dry weight of subsample} \div \text{Wet weight of subsample}) \\ &= (5.00 \text{ g}) * (0.05 \text{ g} \div 0.50 \text{ g}) = 0.50 \text{ g} \end{aligned}$$



**Figure 1. Corn roots colonized by AM fungi are stained for percentage colonization measurement.**