Gridline intersect method using compound microscope

(McGonigle et al., 1990; also in Brundrett et al., 1996)

1)Cut stained roots into 1 cm segments and use fine forceps to arrange the segments lengthwise on a thin layer of PVA mountant on a microscope slide.Gently press a 22 x 60 mm coverslip onto the roots starting at one side and working across to the other side to minimize air bubbles.

2)Flatten the coverslips (overnight or longer) using weight made by filling small vials with b-b's.

3)Use a hairline graticule inserted into the eyepiece of a compound microscope to act as a line of intersection with the roots.At 200x magnification evaluate each intersection for AM fungal structures.There are seven possible, and mutually exclusive, categories of intersections:

p = no fungal structures
q = arbuscules
r = vesicles
s = arbuscules and vesicles
t = coils
u = mycorrhizal hyphae (near but not at arbuscules or vesicles)
v = hyphae not seen to be connected to arbuscules or vesicles (they may or may not below to AM fungi)

Note that mycorrhizal hyphae are always intersected in q, r, s and t, and that mycorrhizal hyphae in u are known to be mycorrhizal because they are seen to be attached to arbuscules, vesicles, or both.

4)Examine 150 intersections for each root sample, scoring each intersection in one of the seven categories.

5)Where a total of G(= p+q+r+s+t+u+v) intersections were inspected, the percentage of root length colonized by arbuscules and vesicles are calculated as:

arbuscular colonization = 100 (q + s/G), and vesicular colonization = 100 (r+ s/G).

6) The percentage of root length infected, which is equivalent to the percentage of root length colonized by mycorrhizal hyphae can be calculated in the intersections of type v can be further sub-divided into those with hyphae that are not mycorrhizal (v1) and those that are mycorrhizal (v2).In this case, the percentage of root length colonized by mycorrhizal hyphae = 100 [(q+r+s+t+u+v2)/G].