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SHORT COMMUNICATION

Biomass of mycorrhizal fungi associated with bean roots

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The presence of mycorrhizal infection of roots is usually confirmed by microscopic examination of stained root segments. This technique is adequate for determining fungal presence or confirming that uninfected controls are, in fact, uninfected, but does not indicate the extent or size of the fungal biomass. The line intercept method of determining percent infection (Ambler and Young, 1977) indicates the length of root infected by mycorrhizal fungi, but again gives no indication of fungal biomass. We have attempted to find a relation between percent root length infected and fungal biomass and to derive an equation to convert the biomass of an easily weighed portion of the fungal biomass into total mycorrhizal fungal biomass for a *Glomus mosseae* symbiosis.

Inoculated faba beans (*Vicia faba* L.) were grown in a sterilized sand:soil (1:1 v/v) mixture with 3 concentrations of added P to produce root systems with different degrees of mycorrhizal infection. After 4–5 weeks growth, six 2.5 cm dia cores were removed from each pot. Three of these cores were used to determine the mass of mycorrhizal vesicles and external hyphae. The remaining three cores were used to determine the mass of internal hyphae. In each case, the roots from the three cores were pooled to give a suitable sample size (Giovanetti and Mosse, 1980). Replication was not possible because of variation between plants.

The biomass of internal vesicles and external hyphae and the percent of root length infected was determined on cores which were individually wrapped in cheesecloth then washed free of loose soil (Mosse, 1959). The roots were picked from the sand remaining in the cheesecloth and placed in 100 ml water. The suspension was sonicated for 2 min (130 W) to dislodge remaining soil and organic particles and most of the external hyphae. The hyphae remaining on the roots were picked off manually under a dissecting microscope and placed in the 100 ml suspension. The suspension was blended for 2 min at high speed, then six 1.0 ml aliquots were removed and filtered through micropore filters (0.4 μm, Nucleopore). The hyphae on the filters were stained with water-soluble aniline blue (0.1%, 20 min) and measured microscopically (×125) using epifluorescence and converted to biovolume assuming the hyphae were cylindrical. Roots grown in the absence of mycorrhizae (control) were treated in an identical manner to determine the effect of other rhizosphere fungi on the technique. Mycorrhizal hyphae could be distinguished from most rhizosphere fungi on the basis of hyphal diameter and appearance, i.e. varying thickness of individual hyphae, aseptate angular branching, bumps on walls and color. Non-mycorrhizal hyphae, while present, were not overly abundant.

The roots remaining in the sonicated suspension were patted dry, weighed, cleared and stained (0.01% acid fuschin in lactophenol). The percent of root length infected was determined by the line intercept method (Ambler and Young, 1977). All the internal vesicles in the root segments were counted and measured microscopically (×125) to determine their number and dimensions. The linear measurements were converted to biovolume assuming the vesicles described spheres or ellipsoids.

Roots from the unsonicated cores were washed and sonicated to free them of soil and external hyphae. The clean roots were patted dry, weighed, dried, ground then blended in 100 ml water for 2 min at high speed. Six 1.0 ml aliquots were removed and filtered onto micropore filters (0.4 μm, Nucleopore). The material on the filters was stained with hot acid fuschin (0.1% in lactophenol). The stained pieces of internal hyphae were measured under the microscope (×125).

The arbuscules proved difficult to measure because they are small organs composed of very thin hyphae. The procedure for measuring the internal hyphae called for high speed blending of the roots. This procedure probably broke the arbuscules into small pieces which could barely be seen by light microscopy, and certainly could not be measured with accuracy. Oil immersion lenses did not have sufficient depth of field to measure the hyphae. Thus, arbuscular masses could not be estimated, and total fungal mass estimates are lower than actual values.

In all cases of biomass measurement, fresh root weights were converted to dry root weights using a factor of 0.1 g dry root g^{-1} fresh root in order to express the fungal biomass on a mg g^{-1} root basis. The dry root weight factor was measured by weighing a sample of fresh roots, oven drying then weighing again. Fungal biovolume measurements were converted to biomass using a conversion factor of 0.35 g cm^{-3} (van Veen and Paul, 1979).

A sample of 900 spores was counted, dried and weighed to obtain an average weight per spore of 0.22 μg. This value was used in conjunction with spore numbers produced by the experimental plants to determine the spore biomass. The spore biomass was converted to a mg g^{-1} root basis using the total root weights of the individual plants.

The four components of the fungal mass were added together to give the total mycorrhizal biomass at each level of infection. Seven levels of infection were studied, ranging from 15.6 to 62.3%.

Mycorrhizal fungal biomass, as a proportion of total root mass, increased as the degree of root infection increased (Fig. 1). At low levels of infection (16%), the fungi comprised less than 0.5% of the total root mass. At relatively high levels of infection (62%), over 5% of the total root mass was composed of fungal material. All fungal components contributed to the increase; however, the biomass of vesicles increased by the largest degree. The variation that can be expected in biomass measurements can

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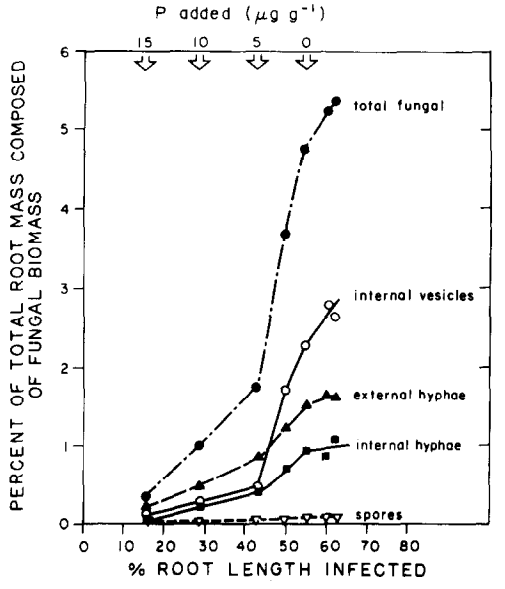

Fig. 1. Biomass of *Glomus mosseae* in and on roots of faba beans (4- to 5-week-old) with varying degrees of infection.

be estimated by comparing biomass values obtained for plants with 61 and 62% mycorrhizal infection. Both plants were from the same treatment. However, a third plant in the same treatment had a lower percent of root length infected. Variation in fungal biomass estimates for any of the other points in Fig. 1 can therefore be expected to be similar.

Analysis of mycorrhizal infection by the line intercept method does not give an indication of mycorrhizal biomass within a root, only of the proportion of the length of root system containing mycorrhizal fungal structures. Regression analyses of the biomass data showed that the biomass of internal or external hyphae, as determined microscopically, was highly correlated with the total mycorrhizal biomass of a root (Fig. 2). The percent infection values were not as well correlated and are, therefore, less accurate measurements of biomass size. This measurement took into account the amount as well as the occurrence of hyphae in each infected root section.

Mycorrhizal fungal biomass was measured using 4- to 5-week-old *Vicia faba* plants. Plants at this stage of development had symbionts that were developing rapidly and spreading through the host root system. Since mature spores and thick-walled resting internal vesicles were not very numerous, inactive biomass could be considered to be minimal. Thus, the biomass present during this stage of host–fungus development was all metabolizing and so constituted the active fungal component. Older root systems may have larger amounts of fungal biomass; however, much of the fungal biomass of these roots will consist of resting internal vesicles and unattached spores, both of which no longer utilize host metabolites.

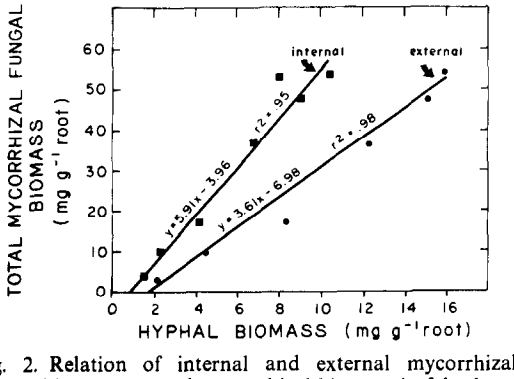

Fig. 2. Relation of internal and external mycorrhizal hyphal biomass to total mycorrhizal biomass in faba bean roots (4- to 5-week-old).

The values obtained for mycorrhizal biomass are lower than those obtained by Hepper (1977) using a colorimetric method. However, in her studies, plant roots were 95–100% infected with fungal symbionts. Our estimates of fungal mass did not include plants with infection levels above 62%, so biomass estimates are lower.

Fungal biomass estimates had to be calculated from microscopic measurements. Similar techniques used for soil fungi yielded almost complete recovery of all added hyphae (Paul and Johnson, 1977). A shortcoming of the microscopic technique is that very fine fungal structures within the host, such as arbuscules, could not be accurately measured. Since the hyphae composing the arbuscules are very thin, their volume is small despite their large surface area. Cox and Tinker (1976) found arbuscules comprised *ca.* 1% of the volume of an infected root segment, however, arbuscules are only present in newly colonized areas of the root. Thus, while locally they may comprise a substantial proportion of the fungal mass, their overall contribution is much less. Nevertheless, our values for fungal mass are underestimates because much of the arbuscular tissue was not measured.

Sanders *et al.* (1977) found a direct relationship between the weight of external mycelium of four endophytes and the length of infected root.

We found a relation between external mycelial weight and total fungal weight. Since the total fungal weight increased with increasing percent of root length infected, our results agree with those found previously.

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