

Drop size analysis at various locations on a  $m^2$  area under the single-nozzle simulator gave a mean volumetric drop diameter of 2.25 to 2.5 mm, with a range of 1.75–1.0 to 2.5–2.75 for the 26 measurements taken. Kinetic energies calculated on the individual drop size distributions ranged from 18.9 to 28.1  $J\ m^{-2}\ mm^{-1}$  (Fig. 2), with an overall mean of 23.1. There did not appear to be any trend in the spatial distribution of the kinetic energy values, indicating a relatively random distribution of energy over the plot area. Both the range of values obtained and the mean are within the limits reported for natural rainfall (Hudson, 1972; Carter et al., 1974).

The advantages of the simulator design presented here are chiefly the low cost and ease of operation of the units. In performance the unit compares favorably with other small ( $m^2$  plot) simulators utilizing clutch-driven oscillating nozzles (Meyer and Harmon, 1979) or rotating disks (Bubbenzer et al., 1985). The major expenses incurred in construction are the solenoids (\$150 each) and the transformer (\$200). All other parts are stock hardware items that can be assembled with little technical assistance. In operation the solenoids have proven to be reliable, with little tendency to jam or clog. The single-nozzle unit is mounted in a greenhouse 3 m above a pair of 0.3- by 1.0-m runoff pans, where replicate rainfall events have shown <10% variation in runoff and soil loss both between pans and between separate events. In the field the three nozzle unit requires less than an hour's set-up time, and is light enough to be moved intact from plot to plot by two persons. Two of these units can be alternately operated, one actually spraying while the other is being readied on an adjacent plot, providing nearly continuous data collection with a crew of 2 to 3 persons.

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## COMPARISON OF TWO METHODS FOR QUANTIFYING EXTRARADICAL MYCELIUM OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI<sup>1</sup>

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### Abstract

Extraradical mycelium of *Glomus mosseae* (Nicol. & Gerd) Gerd and Trappe, a vesicular-arbuscular mycorrhizal (VAM) fungus, was assessed by two quantitative methods using subsamples of the same soil. Fungal biomass was determined by a chitin assay and the length of fungal hyphae in the soil was obtained by direct microscopic measurements. Total hyphal length, including hyphae <5  $\mu m$  in diameter, was not correlated ( $P > 0.05$ ) with total soil chitin; however, the length of hyphae  $\geq 5\ \mu m$  in diameter correlated significantly ( $P < 0.01$ ) with VAM-fungal biomass. There is a strong relationship between these methods for the determination of the extraradical VAM-fungal mycelium. The two methods permit the calculation of a "specific mass" ( $\mu g/m$ ) for VAM-fungal hyphae. Considerations for using either method to quantify extraradical VAM-fungal mycelium are discussed.

*Additional Index Words:* chitin assay, *Glomus mosseae*, *Glycine max*, hyphal length, soybean.

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THE MYCELIUM OF VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) fungi colonizes plant roots and extends into the surrounding soil. The fungi interact with the host plant and the soil microflora and have an impact on both by mediating a bidirectional flow of nutrients (2, 19, 20): minerals to the host, C to the soil. An assessment of the development of the intra- and extraradical structures of VAM fungi is therefore important to evaluate their contributions to plant growth and soil structure. However, the ease with which the extent of root colonization may be quantified is matched by the difficulty of determining the extent of soil colonization (2, 3, 9, 13, 21). Consequently, information on the extraradical mycelium is scant, and results may be biased by the diverse methods used (5, 9, 15, 18, 21).

As uptake organs of nutrients from the soil, serving as extensions of the host root, extraradical VAM hyphae have been thought to be of primary importance in bringing about the VAM growth response (9, 11, 17). However, evidence of VAM growth effects that are not directly linked to improved mineral nutrition due to VAM-fungal colonization is now accumulating (10, 14, 16). Thus, the relationship between the relative development of mycelia inside and outside the root (1, 7, 9) and their impact on host-plant growth (2, 6) is largely speculative at this time. In order to understand the nature of the growth effect, i.e., mineral or water uptake by the extraradical hyphae, and/

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or metabolic processes initiated by the plant in response to the infection of its roots by the intraradical mycelium, it is important to quantify both extra- and intraradical mycelia whenever possible. An evaluation of the merits and shortcomings of methods to quantify extraradical mycelia has been presented by Ahmadsad (3), who also proposed a procedure based on the chitin assay (12), comparing washed VAM roots with or without extraradical hyphae as most appropriate. The purpose of our report is to offer a comparison of the two methods which have been used in our laboratory and to determine if the results obtained by these methods are mutually reinforcing.

### Materials and Methods

Soybean [*Glycine max* (L.) Merr. cv. Hobbit] plants were colonized by the VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd and Trappe, and grown in pot cultures in a heavy silt-loam soil/fine sand (2:1, w/w) mix for 48 d. Three groups of plants (six replicates of each) were exposed to drought cycles of differing severity by rewatering at soil water potentials of  $-0.05$ ,  $-0.3$ , and  $-1.0$  MPa under controlled conditions. Differences in the development of both host and endophyte as a result of stress were noted. The biomass and hyphal length of VAM fungi were determined by the chitin assay (12) and by microscopic measurements (5), respectively. Total fungal hyphal length, including that of non-VAM, saprophytic fungi, was also determined. Hyphae  $> 5$   $\mu\text{m}$  in diameter were adjudged to be predominantly those of the VAM fungus *G. mosseae*.

Lengths of hyphae both greater and  $< 5$   $\mu\text{m}$  in diameter were determined in three subsamples of 1.0 to 1.5 g each of soil from each of the six replicates of the three treatments. Each subsample of soil used for estimating lengths of hyphae was placed in 50 mL of a pH 7.0, phosphate-buffered salt solution. The soil-solution mixture was blended for 30 s at full speed in a Virtis-23 blender. A 1.0-mL sample of this mixture was placed in a small screw-top test tube that contained 0.5 mL of 0.05% trypan blue in lactophenol. The contents of the test tube were mixed and heated in a 70°C water bath for 20 min. Slides were prepared to accommodate a known volume of the solution containing the hyphae. The contents of each tube were thoroughly mixed prior to sample removal. The slide containing the sample suspension was placed under a compound microscope, and three transects per slide were observed for hyphae. Three such slides were prepared for each soil sample. Hyphal lengths and thicknesses were determined with the aid of an ocular micrometer. By knowing the volume of suspension within each transect, the volume of the original suspension, and the dry weight of the soil sample, the lengths of hyphae per gram dry weight of soil were calculated.

The determination of VAM-fungal biomass, as originally described by Hepper (12) and adapted to the quantification of extraradical mycelia by Pacovsky and Bethlenfalvay (15), was further modified. Soil from non-VAM comparison plants was used to construct a standard curve based on the addition of purified chitin to the soil. Four 30-g samples of soil were swirled in 0.3% (w/w) sodium hexametaphosphate [ $(\text{NaPO}_3)_6$ ] for 10 min to break down aggregates. Fungal structures contained in the resulting colloidal suspension were floated in a water/glycerol (1:1, v/v) mixture in a bubbling apparatus described by Furlan and Fortin (8). After settling for 5 min, the suspensions were siphoned off and washed on a 43- $\mu\text{m}$  screen to eliminate small clay particles. The residues were vacuum-filtered and autoclaved together with the filter paper after adding 0, 300, 600, or 1200  $\mu\text{g}$  of chitin (purified, crab shell, Sigma Chemicals, Inc.). The procedure was replicated three times, and six colorimetric determinations were run

for each level of chitin within each of the three replications. Each of the four points of the standard curve was thus based on 18 data points. The point without chitin addition represented the level of chitin in the soil due to organisms other than VAM fungi. Soils of VAM plants were prepared as described above and were evaluated for chitin content by means of the standard curve. Fungal biomass was calculated from the chitin content of extraradical mycelium (85  $\mu\text{g}$  of chitin/mg dry weight of fungus), as described elsewhere (6).

The relationship between hyphal length and biomass of the VAM fungus was evaluated by linear regression analysis for the six replicates of the individual stress treatments and for all 18 biomass/hyphal length combinations (three treatments, six replicates each). In comparison, similar evaluations were made for total soil chitin vs. total fungal hyphal length.

### Results and Discussion

The validity of the methods used here in measuring the extent of the VAM-fungal mycelium in soil depends on a number of assumptions. The main assumption in determining extraradical fungal biomass by the chitin assay is that non-VAM comparison soils, otherwise subjected to the same conditions as the VAM soil to be investigated, contain chitin-containing organisms (other than VAM fungi) not significantly different from those of the VAM soil. Some evidence for this is provided by Ames et al. (4) who showed that the microbiota in VAM and non-VAM comparison soils is similar when both soils are treated with a wash prepared from VAM-inoculum soil and sieved (43  $\mu\text{m}$ ) to free it from VAM propagules. This permits the use of non-VAM soil as an internal standard in constructing a chitin-based standard curve. Another assumption, relevant to absolute biomass determinations, but immaterial for treatment comparisons, is the quality and the source of chitin used in establishing the standard. At present, "purified" crabshell chitin, provided by commercial outlets, is the best available, but probably has a variable composition. The preparation and subsequent chemical analysis of 24 samples requires 2 work days, and is greatly influenced by the nature of the soil assayed. Fine-textured soils free of organic matter (such as the one used in this experiment), whose particles may be almost completely eliminated by the final washing of the sample through a 43- $\mu\text{m}$  sieve, are probably most amenable to the chitin assay. However, many determinations may be necessary to attain acceptable confidence intervals. The applicability of these procedures in a wide range of soils of different characteristics remains to be established.

The hyphal-length determination is more time consuming (4 d/24 samples) but provides a direct assessment of fungal hyphae. The assumption here of a 5- $\mu\text{m}$  hyphal diameter as the dividing line to differentiate VAM from non-VAM fungi is arbitrary. It rests on experience based on many observations, and readily admits the likelihood of overlap in both directions: some VAM-fungal hyphae may be smaller, some non-VAM hyphae  $> 5$   $\mu\text{m}$  in diameter. Support for this number is provided by Ames et al. (5).

The present data confirm the above assumptions. The significant linear correlation between length of fungal hyphae  $> 5$   $\mu\text{m}$  in diameter and VAM-fungal biomass determined by the chitin assay indicates that

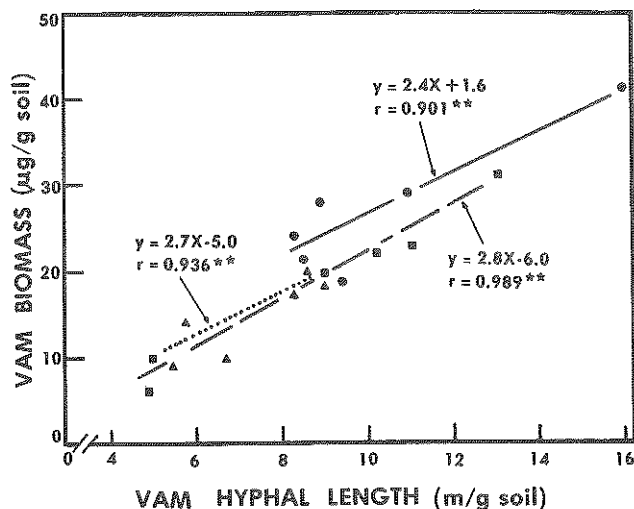


Fig. 1. Biomass and hyphal-length relationships of extraradical VAM-fungal mycelia. Mycorrhizal plants were grown at three levels of drought stress (●—●—●, -1.0 MPa; □—□—□, -0.30 MPa; △·····△, -0.05 MPa). Only hyphae >5  $\mu\text{m}$  in diameter are represented. Regression data of all points are:  $y = 2.7x - 5.5$ ,  $r = 0.936$ ,  $P < 0.01$ .

the two methods quantify the extraradical mycelium (Fig. 1). The lack of such correlation between total hyphal length and soil chitin due to all organisms further confirms this interpretation (Table 1). Correlation coefficients relating total soil chitin and total hyphal length were  $r = 0.558$ ,  $r = 0.198$ , and  $r = 0.141$  for the -0.05, -0.30 and -1.0 MPa treatments, respectively, and  $r = 0.449$  for the composite of all values. These  $r$  values were not significant ( $P > 0.05$ ).

The two methods employed here in assessing the extraradical VAM-fungal mycelium permit the calculation of a specific measure: hyphal biomass/hyphal length (Table 1). This measure could be useful in evaluating the fitness or fragility of the mycelium. This in turn may be a function of age, nutrition, soil texture, host compatibility, or other environmental conditions and may affect the distribution (1) of hyphae in the soil. In the present case, a significantly greater biomass/hyphal length ratio was recorded under severe drought stress than under moderate or no stress (Table 1). Since the biomass indicator is based on a property of the cell wall (chitin), one may infer that the hyphal or spore cell walls were thicker or the hyphae themselves greater in diameter under drought stress than in the absence of stress. Such information has an impact on VAM-fungal taxonomy which is partially based on spore wall thickness. Alternatively, soil chitin content may be influenced by drought stress which induces many soil microorganisms to produce resting stages with different wall compositions (i.e., chitin). Also, the ephemeral lateral branches of VAM-fungal hyphae may collapse under water stress. This would have a combined effect of increasing soil chitin while decreasing measurable hyphal length.

Preference for one of the two methods described here will depend largely on the suitability of the soil to be analyzed for the chitin assay. This method is the less time consuming of the two. However, some of its many steps may introduce a considerable degree of

Table 1. Fungal characteristics in soil as a function of drought stress. Numbers are means and standard errors of six replicates and represent total or VAM-based soil chitin and total VAM (>5  $\mu\text{m}$  diam) fungal hyphal length. Specific weight (dry weight/hyphal length) applies to the extraradical mycelium of the VAM fungus *Glomus mosseae* only.

Parameter	Stress (MPa)		
	-0.05	-0.03	-1.00
Chitin ( $\mu\text{g/g}$ soil)			
Total	1.9 $\pm$ 0.2	2.2 $\pm$ 0.4	2.9 $\pm$ 0.3
VAM	1.3 $\pm$ 0.2	1.6 $\pm$ 0.3	2.3 $\pm$ 0.3
Hyphal density (m/g soil)			
Total	59.2 $\pm$ 2.6	6.6 $\pm$ 1.7	71.6 $\pm$ 3.4
VAM	7.0 $\pm$ 0.8	8.8 $\pm$ 1.3	10.4 $\pm$ 1.2
Specific weight ( $\mu\text{g/m}$ )			
VAM-fungal hyphae	2.0 $\pm$ 0.2	2.0 $\pm$ 0.2	2.6 $\pm$ 0.2

variability not derived from the treatments. Procedures must therefore be carefully standardized. The microscopic method has the advantage of being applicable to any soil, but the very large number of repetitive measurements make it more difficult. Thus, the nature of the growth medium, equipment availability, and operator training will influence the choice of method.

With one exception (9), all methods of quantifying extraradical VAM fungal mycelia are variations of the two methods compared here. It is therefore reassuring that their results are mutually reinforcing. Since they describe different aspects of the development (biomass vs. hyphal length) both should be employed for a more complete description of the mycelium. However, new methods to differentiate between live and dead hyphae are also urgently needed.

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## A SIMPLE DEVICE FOR SECTIONING SATURATED SOIL CORES UNDER LABORATORY CONDITIONS<sup>1</sup>

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### Abstract

The laboratory technique described for obtaining thin horizontal sections of prepared saturated soil cores is very simple, quick, and employs a one-piece assembly. It can be used effectively for monitoring various chemical, electrochemical, and physical parameters of soils incubated under flooded conditions. An average coefficient of variation in sample weights of 4.79% was obtained with this technique for sectioning of the cores prepared in the laboratory, which compares well with results obtained using other more complex apparatus.

*Additional Index Words:* cross sectioning method, flooded soil, plastic canister, distribution of ions, soil incubation.

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IN MOST LABORATORY STUDIES, attempts to understand the transformations and vertical distribution of substances added through agricultural chemicals require incubation of soils under flooded conditions. Incubation is also needed to monitor various chemical, electrochemical, and physical parameters. After a desired period of incubation, the wet soil core is horizontally sliced into different sections followed by analysis of the particular substance or parameter. Brown et al. (1964) developed a technique in which the soil core was quick frozen and sliced into thin sections with a microtome. A similar set-up was used by Patrick and DeLaune (1972) for the characterization of the oxidized and reduced zones in a flooded soil. However, freezing of the soil may have caused expansion and disruption of the wet soil and could have brought about both chemical and biological changes. Later on, Reddy and Patrick (1976) and Savant and De Datta (1979) developed techniques for collecting and sectioning of undisturbed saturated soil cores obtained from flooded rice paddy soils. The method proposed by Reddy and Patrick was used for sectioning of wetland soil cores prepared in the laboratory as well. However, the technique developed by Reddy and Patrick is not so simple for the following reasons: (i) it requires a paraffin-petroleum jelly base, (ii) an A.O. Spencer no. 860 sliding microtome is required for extruding the soil, (iii) other components like a metal rod, a wide pan, and clamps are required for the assembly, and (iv) there is a little compaction of the soil.

The technique described in this paper for obtaining thin horizontal sections of a wet soil is very simple, quick and above all uses a one-piece assembly. It can be used effectively for monitoring various chemical, electrochemical, and physical parameters of flooded soils incubated under the laboratory conditions.

## Materials and Methods

### *Preparation of Cores*

The device used for incubating soils under flooded conditions followed by sectioning into horizontal layers consists of a plastic canister fitted with a base, driver, pan, spindle, and lid. The specifications of the canister are shown in Fig. 1. The canister is 37 by 92 mm, having thickness of driver, pan, and base of 8, 10, and 12 mm respectively. The base, driver, and spindle are connected with the main body of the canister. The pan can be moved up or down along the spindle by rotating the base. We have adapted an assembly that is commercially available as a 56.8g deodorant canister from Gibson Associates Inc., Cranford, NJ. The base of the canister is sealed with plastic or silicon rubber to prevent leaking. The plastic rubber is preferred over silicon rubber as it is easy to peel off. The canister height can be adjusted to accommodate varying amounts of air-dried soil. In the present study, 35-g portions of an air-dried soil (clayey Oxisol from Sumatra) ground to pass through 0.5 mm mesh sieve are placed in a set of canisters followed by incubation under flooded conditions for a desired period of time. Water is introduced from the bottom of the air-dried soil in the canister with the help of a syringe so that entrapped air escapes. During the incubation period, an overlying floodwater depth of about 2 cm is maintained by adding water. Incubation temperature up to 55°C will not affect the canister operation.

### *Sectioning of Soil Cores*

Before sectioning the saturated soil core the floodwater is carefully decanted out with a syringe. Then the plastic or