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QUANTIFYING VESICULAR–ARBUSCULAR MYCORRHIZAE: A PROPOSED METHOD TOWARDS STANDARDIZATION*

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Summary

A standard method for the quantification of root colonization by vesicular–arbuscular (VA) mycorrhizal fungi is needed. From the examination of roots from three different host species, the estimation of the percentage of the length of root segments containing VA mycorrhizal fungal structures was found to be more accurate than the determination of the percentage of root segments with VA mycorrhizal fungal structures. It was no more time consuming, and was not influenced by segment size. Examination of a minimum of seven samples, each with 25 randomly selected 0·5 to 1·0 cm root segments, was needed for confidence limits to be within 10 % of the mean. It is proposed that, for the sake of comparability between studies, this procedure be adopted as a standard method.

Introduction

Mycorrhizal fungi are normal root symbiotic inhabitants which aid plants primarily in uptake of water and mineral nutrients. The degree of exchange between the cortical cells of the host root and the fungal endophyte apparently depends largely on the amount of exchange surface and on the inherent efficiency of the endophyte in acquiring water and nutrients, especially phosphorus and zinc. It is important, therefore, to be able to quantify simply and accurately the amount of fungal endophyte in a root system.

Various methods have been used to quantify VA endophytes in host roots. Becker and Gerdemann (1977) developed a colorimetric method to measure the yellow pigmentation of mycorrhizal roots, and Hepper (1976) used a colorimetric assay to measure conversion of fungal chitin to glucosamine.

The percentage colonization by mycorrhizal fungi has been estimated by other researchers, using the presence or absence of fungal structures in roots, in 1 cm segments (Nicholson, 1955; Read, Koucheki and Hodgson, 1976), 150 μm microscope fields (Furlan and Fortin, 1973), 1 mm grid sections (Davis, Menge and Erwin, 1979), root length increments (Sutton, 1973), and grid intersection points (Ambler and Young, 1977). Methods of measurement have also used estimates of the proportional colonization of root segments (Hayman, 1970) and of entire root

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systems (Ames and Linderman, 1977). Techniques used to measure colonization by VA fungi have recently been summarized by Giovannetti and Mosse (1980).

Some of these methods have a limited application because they only work with certain plant species; others do not accurately assess the percentage of the root length with mycorrhizae, are extremely time consuming, or do not specify the sample size necessary for acceptable precision. Hence, a fast and reasonably accurate method for quantifying endophyte colonization in VA mycorrhizae is needed; such a method is described here. It is recommended that this or some other standard method of measuring infection be accepted so that comparisons can be made between the results of researchers working in different geographical areas or with different plant species.

MATERIALS AND METHODS

Colonization of roots by VA mycorrhizal fungi was assessed on individual plants of Easter lily (*Lilium longiflorum* Thunb.), timothy (*Phleum pratense* L.), and peppermint (*Mentha* × *piperita* L.).

Total root systems of individual plants were cut into 0·5 to 1·0 cm segments and cleared and stained with trypan blue according to the method of Phillips and Hayman (1970). A randomly selected aliquot of stained root segments suspended in lactoglycerin was spread in a Petri dish marked with a 1 cm grid to facilitate scanning, and viewed under a stereomicroscope at 12 to 50×. The proportion of the length of each root segment which contained vesicles, arbuscules, or hyphae of the endophyte was estimated to the nearest 10%. Data were recorded as frequency distributions from samples containing 25, 50 or 100 root segments. The percentage of the root length with mycorrhizal endophytes in the sample was then calculated from the frequency distribution, as shown in Table 1. Colonization was also measured as the proportion of the total number of root segments with vesicles, arbuscules or hyphae, by use of the same root segments as in the previous measurements.

Table 1. *Calculation of the percentage of the root length with mycorrhizal colonization in a sample of 50 root segments (0·5 to 1·0 cm) from a frequency distribution of the percentage of segment lengths with mycorrhizal colonization*

Percentage of segment lengths colonized	Frequency	Frequency × percentage	Calculated percentage of root length colonized
0	8	0	
10	3	30	
20	2	40	
30	2	60	
40	1	40	
50	3	150	3180/50
60	1	60	
70	3	210	
80	2	160	
90	7	630	
100	18	1800	
	50	3180	63·6%

The percentage of the root length with mycorrhizal fungus structures and the percentage of root segments with mycorrhizal fungus structures of each plant and associated confidence intervals were calculated for increasing numbers of samples of root segments.

Results

For all host plants tested, the results obtained by quantifying colonization by the percentage root length with mycorrhizal fungus structures were different from those obtained by counting the percentage of root segments that were mycorrhizal (Table 2). On the heavily colonized Easter lily plant, few root segments without

Table 2. *Estimation of mycorrhizal colonization of individual plants of Easter lily, peppermint and timothy using the percentage of the root length colonized compared to the percentage of the number of root segments colonized*

No. of samples per plant*	Percentage colonization					
	Peppermint		Easter lily		Timothy	
	Root length	Root segments	Root length	Root segments	Root length	Root segments
2	64·8±35·4†	84·0±50·7	67·0±67·0	100·0 (±0)	37·2±62·8	68·0±68·0
3	61·5±15·9	85·3±11·6	69·1±24·0	100·0 (±0)	33·6±13·8	70·6±23·0
4	62·9±9·5	87·0±8·0	72·3±16·2	100·0 (±0)	34·1±7·4	69·0±13·1
5	63·4±6·5	85·6±6·7	71·8±11·1	100·0 (±0)	33·6±5·1	66·4±11·4
6	62·8±5·1	85·3±5·1	73·5±9·5	100·0 (±0)	32·5±4·7	66·0±8·6
7	62·3±4·3	84·0±5·2	73·1±7·7	99·4±1·4	31·9±4·1	64·6±7·8
8	62·5±3·6	83·5±4·5	73·1±6·4	99·0±1·6	32·6±3·7	65·0±6·6

* Samples contained 25 segments, 0·5 to 1·0 cm in length.
† Confidence limits ($P = 0·05$).

Table 3. *Estimations of mycorrhizal colonization of individual peppermint plants from various Oregon locations, that compare the percentage of the root length with mycorrhizae and the percentage of the number of root segments with mycorrhizae*

Plant location	Colonization (%)	
	Root length	Root segments
Harrisburg, field-grown	24·4±3·6*†	75·0±7·5
Independence, field-grown	54·2±3·9	72·5±4·2
Corvallis, greenhouse-grown	62·0±3·2	87·0±5·6
Cheshire, field-grown	62·5±3·6	83·5±4·5

* Figures represent the means of eight samples containing 25 segments, 0·5 to 1·0 cm in length.
† Confidence limits ($P = 0·05$).

mycorrhizae were found, but many were not mycorrhizal over their entire length. Colonization as indicated by the percentage of the root length with mycorrhizae differed between peppermint plants collected at three different field locations and one greenhouse-inoculated plant; some of these differences were not detected by measuring the percentage of root segments with mycorrhizae. The plant collected at Harrisburg, Oregon had a lower percentage of the root length with mycorrhizae

than the plant collected at Independence, Oregon. However, the percentage of the root segments with mycorrhizae of the two plants did not differ significantly (Table 3).

With a sample size of 25 or 50 peppermint-root segments, seven samples per plant were sufficient for 95 % confidence limits to be within 10 % of the mean. With 100 segments per sample, five samples were sufficient for this degree of precision (Table 4). With Easter lily and timothy, eight samples of 25 segments each were sufficient to approximate this degree of confidence (Table 2).

Table 4. *Estimations of mycorrhizal colonization of a peppermint plant by the mean percentage of the root length with mycorrhizae, as influenced by sample size and number*

No. of samples per plant	Number of segments per sample		
	25	50	100
2	66·2±22·7*	58·2±58·2	58·7±54·6
3	63·1±14·2	54·3±21·4	58·1±10·9
4	63·0±7·4	55·5±11·8	57·2±6·4
5	60·2±8·6	55·5±7·9	57·2±4·4
6	59·7±7·0	55·3±6·1	56·8±3·4
7	59·1±5·9	56·1±5·2	56·3±3·1
8	58·9±4·9	55·8±4·4	56·5±2·6

* Confidence limits ($P = 0{\cdot}05$).

DISCUSSION

The methods most commonly used to assess VA mycorrnizal fungus colonization are based on the determination of the percentage of variously sized root segments which contain VA mycorrhizal fungus structures. However, the percentage of root segments containing mycorrhizal fungus structures overestimates the extent of colonization, because a segment which is counted as positive may not be mycorrhizal for its entire length, whereas those which are counted as nonmycorrhizal are completely nonmycorrhizal. This inaccuracy is magnified as segment size increases, because with larger segments there will probably be a decrease in the proportion of the length of the segment which is colonized.

Determination of the extent of VA mycorrhiza colonization by estimation of the percentage of the length of each segment which is colonized does not have the inherent inaccuracy discussed above. It takes no more time than to determine the percentage of segments with colonization. Moreover, it is probably applicable to most vascular plant species because it was used successfully on graminoid, fleshy, and fibrous root systems. At very high or low levels of infection it may be a more sensitive measurement of infection than the percentage of root segments with mycorrhizae, because almost all or almost none of the root segments are colonized. In addition, this method can be used to compare samples of root segments of dissimilar size and shape, because it estimates the mean extent of colonization. With the three species tested here, eight samples of 25 segments each were sufficient for 95 % confidence limits to be within approximately 10 % of the mean.

For these reasons, the percentage of the root length with mycorrhizal colonization is recommended as a standard measurement of VA colonization. Use of this as a standard measurement of colonization should appeal to most researchers faced with

choosing a method most appropriate to their needs (Giovannetti and Mosse, 1980) and would facilitate a comparison of the results of researchers working at different localities or with different plant species.

While we recommend the standardized use of the method described here, we still recognize its shortcomings in relation to the activity of the mycorrhizal fungi being assessed. Such a method subjectively describes the extent of colonization in a two-dimensional plane rather than on a more appropriate volume basis which would be much more difficult to estimate (Strzemska, 1975). Furthermore, the assay does not take into account the hyphae external to the root that extend into the soil matrix and which are conceivably even more important than the endophytic structures. Possibly the greatest limitation, however, is the problem of using morphological features as an indication of fungal activity rather than employing a more direct measure such as phosphate uptake. Further work is needed to determine how well correlated morphological indicators are to mycorrhizal fungal activity.

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