QUANTIFICATION OF VESICULAR-ARBUSCULAR MYCORRHIZAE IN PLANT ROOTS

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INTRODUCTION

Vesicular-arbuscular (VA) mycorrhizal fungi have received considerable attention in recent years. Plant scientists have tried to clarify and describe the complex processes by which plant growth is affected by these symbiotic fungi. Until recently, the majority of workers involved in VA fungal research had extensive training in either plant pathology or mycology and were familiar with biological techniques used in assaying material for fungal colonization. These mycologically trained workers could easily adapt standardized mycological assay procedures to suit specific needs for research on VA mycorrhizal fungi. Currently, many plant scientists initiating studies involving VA mycorrhizal fungi have little on no formal training in mycological staining techniques, so there is a need for standard assay procedures that require minimum modification. Such procedures have an additional benefit over a myriad of modifications — scientists working with VA mycorrhizal fungi/plant interactions will have a reference with which to more readily compare research studies.

Whenever there is a mycorrhizal variable in an experiment, quantification is needed to determine the degree and intensity of root colonization of the plants. One cannot assume, even when endomycorrhizal inoculum from pot cultures are added to properly fumigated and well aerated soil, that plants grown in newly infested soils will become colonized. If field soil represents a part of the growth medium mixture, a VA mycorrhizae assay is desirable to determine if treatment response is affected by sporatic mycorrhizal colonization among treatments or plots, even when an endomycorrhizal variable is not an integral part of the plant nutrient study. The primary purpose of any assay for VA mycorrhizae is to establish whether roots are colonized and to determine the degree of development of mycorrhizae within the root system. In addition, depending on the purpose of a specific study, quantification of colonization may necessitate enumeration of the fungal morphological structures (vesicles, arbuscules, spores, mycelium, pelotons) present in the root system, particularly in studies describing physiological or ecological relationships of mycorrhizal development in relationship to plant growth.

The Phillips and Hayman (12) procedure for clearing and staining roots for rapid assay of mycorrhizal colonization represents a major breakthrough in VA mycorrhizal research. This gave plant scientists ready access to a technique that was specifically adapted to VA mycorrhizal research; it was less time consuming than embedding and sectioning and worked well for a wide range of host plants. This procedure quickly became the most commonly used clearing and staining procedure in mycorrhizal research. However, it had a serious drawback that was shared with other published procedures (4,6,11). The phenols or saturated chloral hydrate used in these staining and destaining procedures are hazardous. The fumes from these chemicals are highly toxic at room temperature and heating them — even under laboratory hoods — can result in adverse side effects. In many cases, especially in underdeveloped countries, and some field locations in technically advanced countries, facilities for confining or exhausting these toxic fumes are inadequate. Kormanik and others (9) published a procedure similar to that of Phillips and Hayman's (12) in which they eliminated the toxic phenols or chloral hydrates; their procedure has been equally effective in VA mycorrhizae clearing and staining for a wide range of host species. Either method, used with caution, is adequate for quantification of mycorrhizal colonization for most VA mycorrhizal assays.

Any suitable assay procedure first requires proper selection of susceptible feeder roots. The primary site for VA mycorrhizae to develop is in the cortical region of the terminal feeder roots which is the most active site for nutrient uptake. As roots mature, the cortex ruptures and is sloughed off and, thus, mycorrhizae are seldom observed in older, less succulent plant roots. Fine terminal feeder roots are left in the soil if considerable caution is not taken during the excavation of plant root systems. If root systems are improperly excavated, roots undergoing secondary growth will constitute the greatest portion of the sample, resulting in a significant under estimation of the percentage of roots colonized by mycorrhizal fungi.

Root Sample Collections

Roots can be collected at any time of the year for mycorrhizal assay and need not be immediately processed in the laboratory. Regardless of whether the host plant is annual, perennial, herbaceous, or woody, the fine terminal feeder roots are the primary sites of VA mycorrhizal development. If reasonable care is exerted when roots are excavated, there will be sufficient terminal feeder roots attached to lower order roots that can be used in the sample. A representative sample of the entire root system can be obtained from four or five different portions of the root system and combined rather than obtaining one large sample from a single portion.

To preserve specimens, individual root samples of 1- to 2- grams (fresh weight) are placed in small plastic bags, vials, or reusable Tissue-Tek plastic capsules (Fisher Scientific Co., Pittsburgh, Pa.) with Formalin-Aceto-Alcohol (FAA) killing and fixing solutions. The standard FAA solution made with 50% alcohol with a v/v/v ratio of 90:5:5 is adequate. Tissue-Tek plastic capsules reduce handling considerably because the individual root samples need be handled only twice — once when the samples are placed in the capsule and once when they are placed in the destaining solutions. An additional benefit of these capsules is that treatment codes can be written on them with a No. 2 pencil when the samples are collected and remain visible during destaining. Samples immersed in FAA have been kept up to 2½ years before assay with no adverse effect to the penciled code or the root sample.

Samples collected and preserved in FAA are used primarily for assessing the degree and intensity of mycorrhizal development and are adequate for describing fungal morphological characteristics in the roots. These samples are not suitable for use in nutritional or biochemical analyses. However, tests for obtaining more complex and extensive nutrient or biochemical relationship data should be preceded by root assay of a representive sample for verification of the absence, presence, or degree of mycorrhizal colonization of the roots.

When plants are newly germinated from seed there is a time lag before mycorrhizae are well developed. It takes longer for colonization to occur if spores are the sole source of infesting material. In all probability, there is variability in the time required for different species of VA mycorrhizal fungi to successfully colonize a root system. Where precise measurements or treatments dictate knowing when roots are colonized, sub-sampling at weekly intervals would be advantageous.

Processing Root Samples

An autoclave is often used to heat the clearing and staining solutions because it reduces manhours and provides more consistent results. The clearing and staining schedule presented is that reported by Kormanik and others (9) deleting the toxic phenols from the staining and destaining solutions. A large number of different hosts and VA mycorrhizal fungal species combinations have been successfully assayed using this procedure. We recommend that phenols not be used in the staining and destaining solutions unless experience with specific root specimens show that

elimination adversely affects specimen preparation. Information for using phenols in solutions is provided if one prefers or finds it necessary for optimum specimen preparation. Following is an outline of a clearing and staining procedure employing both an autoclave and a heating procedure for use under a ventilation hood. Also described is a "no heat" method, which can be used under only the most favorable conditions.

Clearing and Staining Specimens

1) Wash root specimens stored in capsules with FAA in tapwater; place in a glass beaker and cover with a 10% KOH solution. A 1,500-ml beaker will hold 45 to 50 capsules. Place petri plate tops on the specimens to keep them submerged. The capsules and KOH solution should not exceed 75 percent of the volume of the beaker to prevent boiling over in the autoclave. Place the specimens in an autoclave at 1.03×10^5 N/m^2 (15 psi) for 10 min (if an autoclave is not available, heat the specimens at 90°C for 1 h in a well-ventilated exhaust hood). The KOH solution clears the host cytoplasm and nuclei and readily allows stain penetration.

2) Pour off the KOH solution and rinse the capsules well in a beaker using at least three complete changes of tapwater or until no brown color appears in the rinse water. DO NOT agitate the capsules vigorously or loose caps may become detached.

3) Cover the capsules in the beaker with alkaline H_2O_2 at room temperature for 10 to 20 min or until roots are bleached. Alkaline H_2O_2 is made by adding 3 ml of NH_4OH to 30 ml of 10% H_2O_2 and 567 ml of tapwater. Three ml of regular household ammonia works well as the NH_4OH source. The alkaline H_2O_2 solution should be made up as needed; it loses its effectiveness even if stored overnight.

4) Rinse the capsules in the beaker thoroughly using at least three complete changes of tapwater to remove the H_2O_2.

5) Cover the capsules in the beaker with 1% HCl and soak for 3 to 4 min and then pour off the solution. DO NOT rinse after this step because the specimens must be acidified for proper staining.

6) Cover the capsules in the beaker with 0.01% acid fuchsin-lactic acid staining solution and autoclave for 10 min at 1.03×10^5 N/m^2 (15 psi). The lactic acid solution consists of 875 ml of laboratory grade lactic acid, 63 ml of glycerin, 63 ml of tapwater, and 0.1 g of acid fuchsin. (If an autoclave is not available, simmer the capsules in the beaker at 90°C in an exhaust hood for 10 to 60 min or until the roots are satisfactorily stained.)

7) After removing from the capsules, place the root specimens and the capsule top in glass petri plates for destaining and mycorrhizal assay. By retaining the capsule top with appropriate codes, the possibility of petri plate tops being inadvertently switched is eliminated. DO NOT rinse specimens after staining because the stain is readily removed from the fungal structure, requiring restaining. The destaining solution is the standard used in Step 6, but, of course, without the acid fuchsin component.

Several changes occur in the staining and destaining steps if lactophenol is used. The lactophenol solution for staining consists of 300 g of phenol, 250 ml of lactic acid, 250 ml of glycerin, and 300 ml of water. Acid fuchsin (0.1 g) is added to the staining solution, but excluded from the destaining solution. If lactophenol is used for staining, it must also be used for destaining. Specimens should be transferred to glycerin after several weeks to prevent excessive destaining. The staining solution made with lactophenol is seldom effective after the second use.

With or without phenols, this clearing and staining technique removes the cellular contents and makes the root opaque, but the VA mycorrhizal fungal structure stains bright red to light pink. During destaining the fungal structures are somewhat more distinct using the phenol solution for several weeks, but after a short period of time equal clarity is attained by the lactic acid solution.

Since the lactic acid staining procedure was first reported by Kormanik and others (9), other benefits of removing the phenols from these solutions have been

observed (Kormanik, unpublished). The staining solutions have worked effectively for up to seven uses, after which they show no evidence of losing effectiveness. Since lactic acid is not volatile at autoclave temperatures, when staining becomes light additional stain can be added rendering it effective. Specimens have been held in the destaining solutions for up to 30 months with no significant loss of stain in the fungal structures. Activated carbon, Grade K-B, can be used to remove the stain from the lactic acid destaining solution which permits recycling. To clear the destaining solution, 10 g of activated carbon per liter of destaining solution is added and left standing overnight. The material is then filtered twice, first with Whatman No. 1 or 2 filter paper to remove the coarse material and larger carbon particles and second, with Whatman No. 42 filter paper to remove the finer carbon particles. This results in a clear solution.

Acid fuchsin is only one of the stains that has been found effective for VA mycorrhizae staining. Many workers prefer trypan blue, Sudan IV, or cotton blue. Of these stains, trypan blue (0.05% trypan blue in lactophenol) has been used extensively for staining many different host/fungus combinations. The use of trypan blue without phenols has not been widely tested. We do not know its effectiveness when used with the above lactic acid staining and destaining procedure. However, as is characteristic of any histological staining technique, slight modifications may be required for different root materials. The major modification will probably involve how long the clearing and staining solutions are heated, which is governed by the succulence of the roots. Too much heat applied to succulent roots results in flaccid or mushy root specimens. When this occurs, reducing time and pressure in the autoclave solves the problem.

No-heat Method.--The no-heat method works effectively only on succulent material. The solutions and procedures are the same as those used when heat is applied, but the time required for each step is greatly increased. If bleaching is required, up to 6 h may be required in unheated KOH. Two hours or longer may be required for the fungal structures to adequately absorb the stain.

Assessment of Colonization after Clearing and Staining

Recently, Giovannetti and Mosse (5) reported techniques for measuring mycorrhizae in roots following clearing and staining. These methods can be broken down into several distinct procedures. They are (i) visual assay, (ii) slide length (estimated length of colonized tissue in root segments of standard length mounted on slides), (iii) slide ± (presence or absence of colonization in the same root segments), and (iv) gridline intersect. These workers pointed out that there is no report comparing the different techniques and, thus, many researchers have developed or modified techniques to meet their own requirements. Their basic conclusion was that over the years the number of root segments per sample has tended to decrease while the number of observations per segment has tended to increase. These workers reported that only rarely have assessments been made based upon a visual assay of large samples containing unspecified numbers of roots.

Giovannetti and Mosse (5) reported that the gridline intersect method had the smallest standard error, followed closely by the visual method; root segments mounted on slides consistently resulted in the largest standard errors with either method. These authors feel that the gridline intersect method is, for most purposes, the most acceptable compromise when all the relative merits and shortcomings of the four systems are considered. The visual method, however, is quite rapid, easily mastered by the novice mycorrhizal researcher, and, because the assay is for a greater number of roots, may be biologically more meaningful to many plant science disciplines than the exacting, time-consuming measurements made by using a limited number of roots mounted on slides. We feel that the four common assay procedures reported by Giovannetti and Mosse (5) fall into two categories: systematic and nonsystematic. Their visual and slide ± methods are viewed as nonsystematic, while

the gridline and slide length methods are considered systematic. We find the term "visual assay" misleading because all the procedures, whether systematic or non-systematic, involved visual observations using either a dissecting or compound microscope. However, both nonsystematic procedures involve root scanning and subjectively give a ± rating to the roots without any attempt at being qualitative. The systematic procedures involve qualitative estimates of the percentage as well as the intensity of colonization within the root sample. For many mycorrhizal assessments, the percentage of roots colonized and the intensity of colonization within the root sample are readily determined by nonsystematic root scanning procedures (with a large, unspecified number of feeder roots). However, if exact measurements and enumeration of fungal components is required per unit root length, then observations can be made on subsamples located on a slide or gridline. For want of a better term, the latter procedure might be considered a "partial systematic procedure." If used properly, the gridline intersect method probably represents the most accurate assay procedure. We do, however, concur with Giovannetti and Mosse's (5) basic conclusion that it is not known which sampling procedure accurately measures the true level of root colonization.

A routine mycorrhizal assay is done on root samples in petri dishes under a dissecting microscope at 40 to 100X magnification. When a more detailed observation of fungal structures is desired, individual root segments must be mounted on a microscopic slide. Determination can then be made with a compound microscope at 100 to 250X magnification.

Once the clearing and staining is completed, it is necessary to differentiate between what is VA mycorrhizae and what is not. Plate 4A illustrates how root colonization appears under low magnification, Plate 4B shows the appearance of internal hyphae and arbuscules at higher magnification, and Plate 4C shows well-developed internal vesicles formed by a Glomus species. With careful root excavation and processing, external vesicles formed by VA mycorrhizal fungal species in the genus Gigaspora frequently are observed; these are illustrated in Plate 1D.

If possible, the first-time viewer of VA mycorrhizae colonization should seek the assistance of an experienced worker. Normally, VA mycorrhizal hyphae are thick and not smooth, hypae of non-VA mycorrhizae fungi in roots are usually thin and smooth, and frequently have septa at uniform spacing along the hyphal strands. However, older VA mycorrhizal hyphae can have septa. The arbuscules are distinct morphological features and not easily confused with anything else. Since arbuscules are delicate structures that are short-lived and readily absorbed, learning to recognize the coarse, nonabsorbed branches is important. One should not confuse these remnants (which can be septate) with non-VA mycorrhizae fungal structures. Identifying and separating VA and non-VA mycorrhizae fungal structures is frequently simplified because non-VA mycorrhizal fungi are not often encountered in VA mycorrhizal colonized roots in quantities that would significantly affect the root assay.

Nonsystematic Method

The evaluation of endomycorrhizal roots using the nonsystematic root scanning procedure can be expressed and quantified in different ways, depending upon the study objective. The primary objective of most studies is to determine the percentage of roots colonized as well as the intensity of colonization within the roots. To do this, roots are spread uniformly in a petri dish with dissecting needles to eliminate clumping and enhance light transmission. Within a sample, the number of susceptible feeder roots colonized determines the percentage of roots with mycorrhizae. The whole-root sample is carefully rotated on the microscope stage and an assessment of the colonized roots is made, usually in broad classes of percentage of colonization. A workable classification used at the Institute for Mycorrhizal Research and Development, USDA Forest Service, Athens, Georgia, is as follows: Class 1, 0-5%; Class 2, 6-26%; Class 3, 26-50%; Class 4, 51-75%; and Class 5, 76-100%. Normally a 3-min examination is sufficient to place the root sample in the appropriate class. When a sample is borderline between two classes,

an assay of five or six random positions in the petri dish may be helpful to properly classify. Because of normal variation in percentage of roots colonized from a given root system, exact placement within a more narrow class range would be time consuming and, possibly, would not be biologically or statistically significant when comparing samples.

Many VA mycorrhizal researchers have found that the nonsystematic root scanning technique is the most rapid method for assessing the percentage of root colonization. Giovannetti and Mosse (5) reported that these subjective techniques gave reliable results when compared with more laborious methods, and that one could attain considerable proficiency with only a few hours' training.

Intensity of colonization is a separate assessment of colonization within roots. This assessment is as important as the percentage of roots colonized, but frequently is not attempted by VA mycorrhizal researchers. Kormanik and others (9) reported three categories for evaluating intensity of colonization. An intensity of 1 is assigned to roots with small colonization sites widely scattered along the roots; an intensity of 2 represents larger colonization sites more uniformly ditributed through the colonized roots, but rarely coalescing; and an intensity of 3 is given when the feeder roots are almost solidly colonized with few easily identified, isolated patches of colonization. A tentative intensity classification frequently can be obtained when percentage of roots colonized is determined, but a final value can be readily obtained by looking at five or six random microscope fields. Using this procedure, one must be constantly aware that only colonized roots are considered in the intensity evaluation because it is independent of the percentage of roots colonized.

While the root scanning nonsystematic procedure is adequate for many studies, it should not be used for quantifying morphological data (vesicles, mycelium, or arbuscules) on fungal infection per sé, but should be supplemented by assaying a subsample using a systematic procedure such as the slide or grid method. The nonsystematic procedure of assaying roots can be, and occasionally should be, checked against a systematic method to help reduce bias. A good time to do this is either when a new person is being trained in mycorrhizal assay or when changes are made in hosts and symbionts in a trial.

Systematic Methods

The most commonly used systematic procedure is the slide method, but the gridline intersect method is probably the most accurate if all theoretical requirements are met (5). Both of these methods are laborious. The stain retention in root segments, even to the naked eye, frequently indicates the degree of VA mycorrhizal colonization, and the accuracy of the assessment using the slide method depends entirely on the unbiased selection of root segments. Selection of root segments can be unintentionally biased.

Gridline Intersect Method.--This method can be used to estimate both the proportion of root length colonized and total root length in the sample. The root sample is spread out evenly in petri dishes that have gridlines marked on the bottom to form 1.27-cm squares. Many VA mycorrhizal researchers using the gridline intersect method ink the grid on a circular piece of acetate and place it on the bottom of the inverted plate top. The bottom portion of the plate containing the sample is then placed inside the top. The grid markings are distinct and the use of acetate is easier than marking grids on a large number of glass petri dishes.

The dimension of the grid squares is important for measuring the total length of roots. If only the percentage of root length colonized is to be determined, the gridlines serve only as a device for systematic selection of observation points. Vertical and horizontal gridlines are scanned and the absence or presence of colonization is recorded at each point where a root intersects a line. Good accuracy for percentage of root length colonized is obtained with this method if at least

100 gridline intersects are tallied. However, for an estimate of total root length, all gridline intersects, or intersects from a predesignated portion of the gridlines, must be counted. Provided that the exact grid spacing described by Giovannetti and Mosse (5) is used, the total number of root/gridline intersects equals the total length (expressed in centimeters) of roots in the petri dish. The theory behind this procedure is given by Newman (10) and Giovannetti and Mosse (5).

Slide Method.--Root segments, each approximately 1 cm long, are selected at random from a stained sample and mounted on microscopic slides in groups of 10. Giovannetti and Mosse's (5) work indicates that from 30 to 100 root segments from each sample should be used for this method. Length of cortical colonization is assessed (at 100 to 250X) in millimeters for each root segment, averaged for each of the 10 segments in a group, and expressed as a percentage of root length colonized. This method gives an assay based on total root length and takes into consideration the percentage of roots colonized, as well as the intensity of colonization. While time consuming, this method quantifies the mycorrhizal development quite well and, because a higher power microscope is used, the fungal structures are distinctive. This procedure may be of limited value when many specimens have to be assayed.

The slide procedure can be simplified by recording only the absence or presence of colonization in each root segment and expressing the results as a percentage of roots colonized. Giovannetti and Mosse (5) refer to this alternative procedure as the slide method ±. We feel this latter procedure is a nonsystematic assay and it is more time consuming than root scanning. This modified procedure appears to be less accurate because fewer roots would be assayed. Giovannetti and Mosse (5) reported that the modified procedure consistently resulted in the largest standard errors in their comparative tests of VA mycorrhizal assessment methods.

Mycorrhizal Assessment of Noncleared Roots

Regardless of which assay procedure is used, evaluating for VA mycorrhizae fungal colonization using clearing and staining methods is rather time consuming. Numerous attempts have been made to develop alternative procedures but none have been entirely satisfactory for a wide range of hosts or fungal structures, therefore, clearing and staining roots remains the primary method used. Colorimetric, chemical, and autofluorescence procedures offer considerable opportunities to improve the accuracy of assessments, as well as reduce the time spent making them; developing and perfecting alternative methods should be encouraged. Examples of these procedures follow, but details should be obtained from the references cited.

Colorimetric Assay.--Endomycorrhizal roots of some host plants develop a yellow pigmentation that diffuses into the water when roots are cut and also disappears rapidly when exposed to sunlight. Daft and Nicolson (3) attempted to assess mycorrhizal colonization of entire root systems by measuring the relative intensity of this yellow water-soluble pigment in tomato roots. The intensity of this yellow pigment was assessed visually by an ultraviolet mercury vapor lamp and was correlated with the intensity of mycorrhizae fungal colonization based on a systematic assay procedure that used paired root systems. The pigmentation assay gave good accuracy under low nutrient conditions when root colonization percentages were high, but basically it was a very subjective assessment. The yellow pigmentation was conspicuous under pot conditions in heavily colonized roots, was not detectable in field-grown plants, and occurred only in certain hosts.

Becker and Gerdemann (2) extracted this yellow pigment from onion roots by autoclaving root systems for 30 min at 121°C. After cooling, the roots were removed and the absorbance of the water extract was read at 400 nm against a water blank in a Bausch and Lomb Spectronic-20 spectrophotometer. If the absorbances could not be read immediately after cooling, the extract had to be kept in the dark to prevent the pigment from fading. They concluded that this colorimetric procedure was a

valid alternative for root assessment for controlled short-term experiments, but not for long-term experiments where phenolic compounds of dying roots could interfere with the absorbance of the water extract.

Herrera and Ferrer (8) indicated that a direct measurement of the amount of endomycorrhizae fungal tissue present in roots can be obtained by clearing and staining roots with trypan blue in lactophenol and then eluting the absorbed stain from the fungal tissue. Supposedly the amount of stain eluted, measured colorimetrically, could be correlated with total colonization. It is possible that contamination by other microorganisms would interfere with measurements. This method has had limited testing.

Chemical Assay.--Chitin is a component in the hyphal walls of VA mycorrhizal fungi. Hepper (7) performed a chitin assay from both endomycorrhizal and nonmycorrhizal roots of several hosts to assess this compound as an assay method. In this procedure, chitin was converted to glucosamine and the absorbance at 650 μm was compared with purified glucosamine. The amount of glucosamine in the root samples was then correlated with the total weight of the root sample. It was also correlated with the percentage of mycorrhizal colonization when varying degrees of colonization were obtained by diluting endomycorrhizal and nonmycorrhizal roots. However, no good correlations were found when glucosamine was expressed per unit dry weight of the external mycelium of colonized roots. Hepper (7) concluded that the lack of correlation occurred because external mycelium from which the internal conversion factor was obtained was not a good measure of internal fungal biomass.

Ultraviolet-Induced Autofluorescence Assay.--Recently, Ames (1) reported a simple procedure for assaying arbuscules in fresh mycorrhizal roots by an ultraviolet-induced autofluorescence procedure.[1/] Colonization is assessed from fresh endomycorrhizal root segments with an epifluorescence microscope equipped with epifluorescent condensers, mercury lamps, exciter filters which passed wave lengths of 455-490 nm, and barrier filters which allowed wave lengths of 520-560 nm to pass to the viewer. Arbuscules autofluoresced in the root segments of all plants tested. Apparently, the species of VA mycorrhizal fungi, the host plant, and the conditions under which the symbiosis occurred did not adversely affect this phenomenon. However, only arbuscules autofluoresced under ultraviolet light; vesicles, spores, and hyphae within or exterior to the root did not. Although fluorescent intensity varied somewhat with the host, newly developed, highly branched arbuscules could be readily distinguished from the older collapsed arbuscules with their smaller, clumped appearance. Although only arbuscules can be detected with this procedure, it is potentially important because the same root segments can be used for determining the presence of VA mycorrhizae and can be used for either biochemical or nutritional assays. If further testing of this procedure reveals autofluorescence of arbuscules to be a reliable and consistent phenomenon, it could have a significant impact on the development of chemical or colorimetric assay procedures.

DISCUSSION

For the present, it appears that clearing and staining roots will remain the most common procedure used for endomycorrhizal root assays. Although it is time consuming when large numbers of samples must be processed, little specialized equipment is needed. Workers are discouraged from using toxic phenols or chloral hydrate in the staining and destaining solutions because suitable results can be obtained with less toxic materials. In our opinion, trials with different stains are not high priority since acid fuchsin and trypan blue have been proven effective for a wide range of hosts and VA mycorrhizae symbionts.

There has been no strong evidence in the literature favoring either the nonsystematic or the systematic procedures for assaying stained roots. The diverse root morphologies characteristic of the numerous endomycorrhizal host plants may,

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[1/] Personal communication, Robert N. Ames, Natural Resources Ecology Lab., Colorado State University, Fort Collins, Colo.

in part, account for the fact that some investigators prefer a systematic procedure for root assays while others prefer a nonsystematic root scanning procedure. It appears, however, that a partial systematic root assessment is beneficial. In this procedure, large numbers of roots could be scanned for percentage of roots colonized and a subsample would be systematically assayed using the gridline intersect or slide method for determining intensity of colonization or enumeration of fungal components.

Further exploration in colorimetric, chemical, and autofluorescence procedures should be encouraged in an effort to develop more qualitative assay methods without the limitations so evident in published procedures. We need accurate, reliable, less time consuming procedures that will work on a wide range of host plants.

Finally, workers are cautioned on the excessive extension of data obtained from VA mycorrhizal assays. Our knowledge of this symbiotic relationship is not sufficient to state which threshold of root colonization will affect growth under different nutritional regimes. This obvious gap in knowledge probably accounts for poor correlations that have been observed by many researchers when growth is plotted against root colonization percentages. Thus, VA mycorrhizal assessments simply tell us what has occurred under the conditions of the test. We feel it is important to know this.

NOTE: The use of proprietary names in this publication does not constitute an endorsement by the U.S. Department of Agriculture or the Forest Service.

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