

COMMENT

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Modeling arbuscular mycorrhizal infection: is % infection an appropriate variable?

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Abstract Several available models of arbuscular mycorrhizal infection are based on fitting % infection to a logistic curve and then relating the various parameters to biological functions. I suggest here that this direction is misleading. Percent infection is a value derived from the growth of two interdependent but distinct organisms, each of which is seeking to maximize its own growth and survival. I suggest that two-organism models, such as those derived from Lotka-Volterra equations, are more useful for understanding the biology and functioning of mycorrhizae.

Introduction

The determination of % root length infected has been the baseline for measuring arbuscular mycorrhizal activity for several decades. Numerous efforts (e.g., Tinker 1975) have been made to model mycorrhizal activity and to relate physiological responses of the host to mycorrhizae based on % infection. (I use the term infection as opposed to colonization to refer to the presence of the fungus within a root system in order to differentiate between individual root mycorrhizae and migration of mycorrhizal fungi onto a site). My questions here are: (1) what is % mycorrhizal infection, and (2) is this an optimal parameter around which to develop mycorrhizal models?

Percent infection

Percent infection is the proportion of the root length bearing mycorrhizal fungi. This includes root sections with arbuscules or with the entire array of structures (coils, vesicles, hyphae). There are many ways of measuring % infection, ranging from numbers of root fragments (1-cm or 1-mm lengths are commonly used) to

whole root intercepts (infinitely small points on the root). Whereas these approaches give different answers, % infection is really a unique value, independent of the method used, being some function of the total (or relative) root length divided by the length of root containing the fungus. Interpretation of the value becomes difficult when only the composite value is known.

Modeling % infection: the logistic equation

In overall analyses of infection, % infection is often described as a sigmoidal function (e.g., Mosse et al. 1981). In this form, following common tenants of microbiology, a lag, an exponential infection phase, and a plateau are distinguished. The lag represents the time for initial establishment of infection and the plateau is the period during which an equilibrium exists between the rate of root growth and the ability of the fungus to initiate new infections. This pattern was recognized largely on the basis of AM expansion in pots, and was codified into a logistic equation (e.g., Buwalda et al. 1982) where the shape of the curve describing % infection is sigmoidal.

Do the coefficients have biological meaning?

Both Pattinson and McGee (1997) and McGonigle (in press) evaluated forms of the logistic equation whilst attempting to explain the functions of the various coefficients. Despite their usefulness in describing infection development under some conditions, I am concerned that these coefficients have no specific biological meaning. They are simply functions of the curve-fitting exercise. The upper asymptote (C_p), following McGonigle (in press), is simply an observation point (Smith 1974). The other parameters (McGonigle in press) t_i (time of infections), and k (abruptness of the curve) are derived and not biologically explicit factors.

The logistic equation mostly fails as a predictive tool because % infection is not a single function but is the result of two more-or-less interdependent processes: root

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growth and successful fungal invasion. The equation depends on the attainment of an equilibrium value (C_p) but there is no reason to expect equilibrium. I will try to illustrate this using data from our laboratory and to derive an alternative framework within which infection modeling could be developed.

Successful infection

Infected length is that part of a root which contains mycorrhizal structures. It can be shown as arbuscular length only or, more commonly, as total infection, including arbuscules, internal hyphae and vesicles. This latter is an indication of both current and past infections because carbon tends to be stored in vesicles or internal hyphae as an infection matures. It is important to distinguish between the use of total infection and arbuscular length. Often arbuscules are not the only, or even the major, transport structure. In maple forests, coils are prevalent but arbuscules are rare (Klironomos et al. 1993). In other systems, arbuscules are only found over a very short period (e.g. Allen 1983).

In many, if not all cases, penetration of a root is limited to the region behind the root tip (Buwalda et al. 1984). The fungus then occupies a distinct length of the root, which may be a function of the ability to deplete resources such as P (Walker and Smith 1984; Fitter 1991). This length or the number of rooting tips represents the potential infection unit. Mycorrhizal infection may often persist for a very long time (e.g., Merryweather and Fitter 1998). The fungus then either retreats or is eliminated from older roots, which retain only xylem transport structures in place of living cortical tissues. The plant may also eliminate the fungus by a gradual build-up of phenolics, which provide protection against pathogens (Allen et al. 1992). Thus, infection is related to the production of new root tips, the penetration of such new roots, the lifespan of the fungus within the root, and the lifespan of the root itself.

Is infection an integrated function?

Infection of roots by mycorrhizal fungi is subject to two major influences. First, infection is affected by soil resources (largely P or N) in relation to the C gain by the plant. This occurs at the whole plant level. In two separate innovative experiments, Sanders (1975) and Menge et al. (1978) demonstrated that change in whole plant P influenced mycorrhizal infection. Sanders (1975) applied P to the leaves but not roots. Mycorrhizal infection declined as the leaves experienced adequate nutrient levels. Menge et al. (1978) separated roots from a single individual in two chambers containing different nutrient levels. In both cases, increasing foliar P inhibited mycorrhizal formation, even when localized root P uptake was lowered. At the other end of the spectrum, under elevated CO_2 with limiting nutrients, AM infection tended to increase or at least remain equal to that of roots in elevated CO_2 and constant soil resources (Treseder and Allen 2000).

Second, infection is affected by mycorrhizal inoculum amount, composition, and distribution. This means that hyphae capable of initiating infections, or germinated spores searching for uninfected roots, must be present and their amount and distribution must be adequate for new infections. Both hyphae and spores are notorious for their highly variable spatial and temporal distributions (e.g., Klironomos et al. 1999). There have been numerous reports that fungal species infect host plants differentially (see Smith and Read 1997).

Examples

Mycorrhizal infections are mainly dependent on plant habit and environmental conditions. Some plant species produce an extensive root system with many tips, whereas others produce few roots. The result may be highly variable % infection, even when the total amount of mycorrhizal fungus is similar. For example, Indian ricegrass [*Achnatherum* (= *Oryzopsis*) *hymenoides*] has a highly branched root system, whereas sagebrush (*Artemisia tridentata*) has a comparatively slower-growing, taproot root system. Given the same inoculum density, the same mycorrhizal root length resulted in highly variable % infection (Fig. 1).

In a second field study, we studied mycorrhizal responses of *Agropyron spicatum* and *Agropyron desertorum* under different environmental conditions. The total root length and % infection were highly variable (Allen et al. 1989), but the mycorrhizal fungal spore counts and mycorrhizal root length per unit soil volume did not change. This could be explained by rapid root growth during the wet periods, yielding a very low % mycorrhizal infection. Conversely, during dry periods the roots grew more slowly and % mycorrhizal infection was relatively high. In neither case would a logistic model fit the % infection pattern. Many similar examples can be drawn from the literature.

Alternate approach

Percent mycorrhizal infection, therefore, is not really a single function. It is a composite measure of the interaction of plants and fungi faced with limited resources: nutrients in the case of the plant and energy for the fungus. Plants could survive without the fungi in virtually all situations as long as nutrients are available. Nutrients may be limited by both soil characteristics (low total nutrients, bound nutrients) and by competition (high plant densities). Carbon allocation may be limited by the photosynthetic capacity of the plant (inadequate light, CO_2 , or nutrient levels) and plant density, or by the density of the fungi and competition for root-occupation sites (Wilson and Tommerup 1992). I propose that modeling should be in the form of two interactive entities and not a single equation. Smith and Walker (1981) have advocated a similar approach. At least initially, modeling should

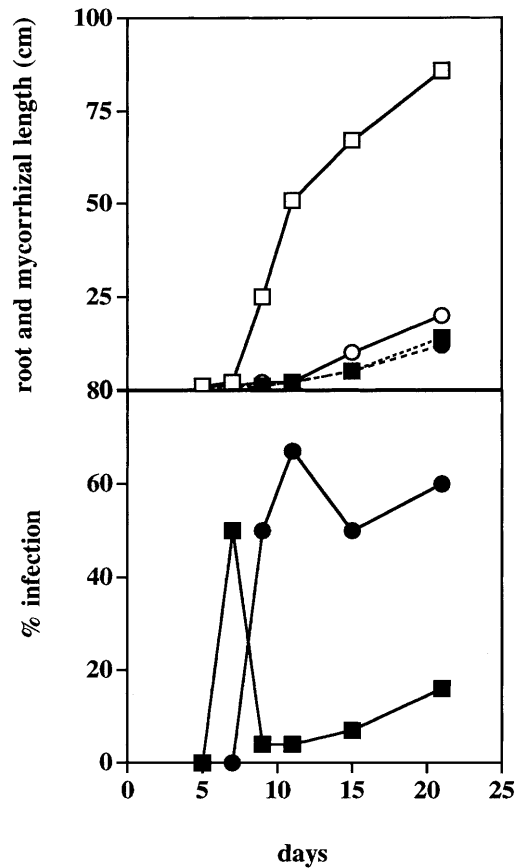


Fig. 1 Development of mycorrhizae in Indian ricegrass and basin big sagebrush. Root and mycorrhizal lengths are shown: Indian ricegrass root length (\square), sagebrush root length (\circ), Indian ricegrass mycorrhizal-infected root length (\blacksquare), and sagebrush mycorrhizal-infected root length (\bullet). As the mycorrhizal root length is virtually identical in both species, but the rates of root growth are very different, the % infection varies rapidly within a host species and with time for both Indian ricegrass (\blacksquare) and sagebrush (\bullet). Data from Friese (1991)

be based on root length and infected root length, or number of root fragments and those that are infected. Percent root infection could be viewed as one possible output. Eventually, the goal would be to link actual infections with the amount and distribution of the fungus present.

The mutualism models utilized are derived from the Lotka-Volterra predator/prey equations (Boucher 1985; Gotelli 1995), where for any population (viewing a root as a population of infectable segments), increasing root length can be described as:

$$N_{t+\delta t} = N_t + B - D.$$

$N_{t+\delta t}$ is the population at time $t + \delta t$, B is the births between time t and $t + \delta t$, and D is the number of deaths between time t and $t + \delta t$. During root expansion, the intrinsic rate of root growth can be reduced to:

$$N_t = N_0 e^{rt}.$$

In this case, r is the growth rate of the roots. In my work, I use 1-mm segments for this modeling exercise. In mycorrhizae, root infection intervals often appear to

average 1 mm (Allen 1982). Walker and Smith (1984) and Fitter (1991) both found this the optimal interval for fungal growth and P uptake.

In the case of the two plants described in Fig. 1, the relative root growth (r) was calculated by measuring intermittent root lengths. For sagebrush $r=0.20/\text{day}$, whereas the initial growth rate (r) for ricegrass was $0.71/\text{day}$. In both cases, the coefficient of determination to the actual root length measured was greater than 0.95.

For the purposes of this analysis, I also looked at mycorrhizal infection. In this case, I assumed that the initial amount of the mycorrhizal fungus is a function of the initial inoculum, which was the same in both cases. Further, I assumed that the subsequent amount of mycorrhizal fungus was related to the prior numbers of infection units. In this case, M is the length of mycorrhizal root segments in millimeters. At this stage,

$$M_t = M_0 e^{\rho t}.$$

In these cases, relative infected root growth (ρ) for ricegrass ($0.201/\text{day}$) and sagebrush ($0.202/\text{day}$) was virtually identical. Again, the coefficient of determination exceeded 0.97 for both plants. These data suggest that ρ is related to the amount of fungus, which may be a function of the initial inoculum plus a fungal growth rate independent of the host species.

Interestingly, during this time and under these conditions, the infection rate of sagebrush was approximately equal to the rate of root growth. In the case of ricegrass, the infection rate was dramatically lower than root growth. This sometimes gives a negative value for change in % infection even as the numbers of mycorrhizal infection units are increasing (Fig. 1).

The factors regulating mycorrhizae can be further evaluated. For example, N is determined by the "birth" of new root segments (B). Similarly, M is a function of the newly formed infections (β). In this particular case, I used the same equations, where $N_{t+\delta t} = N_t + B$ (for mycorrhizae).

Here, B was much higher than β for ricegrass but approximately equal for sagebrush. In other words, the mycorrhizal fungi could not keep up with root growth in the grass but could in the shrub.

As ricegrass continued to grow, the rate of increase in mycorrhizal root length remained constant. However, the rate of root growth began to decline ($r=0.052$) as the volume of soil for exploitation declined. This led to an increase in % mycorrhiza infection. Again, there was no increase in rate of mycorrhizal formation but there was a decline in root growth. Under such conditions, some limit on carrying-capacity, the K value in population growth curves can then be measured (in the case of ricegrass, it appeared to approach 120 cm). It must be noted that K here is the upper limit of resource availability and not an equilibrium and is not to be confused with the abruptness coefficient k of McGonigle (in press). The value of K will depend on factors such as rooting volume available and species. Remember, K must be *measured* (Smith 1974). In the example given in Fig. 1, when K_{root} of ricegrass is approached, ρ is maintained. The changing % in-

fection is determined by the growth rate of the roots and not the fungus. I would predict that root death will increase with time, with an initially very high % infection then a decline in the mycorrhizal roots. No equilibrium will be reached and the % infection measured will be dependent simply on the timing of sampling.

It would be exciting for mycorrhizal ecology to look at the interactions between r and K for the two mutualists as conditions change. Percent infection would become an output variable against which field studies could be conducted. For example, as a root ages, corticle cells suberize and there is an increase in phenolics which make the root uninhabitable for the fungus. Thus, there is a loss of active infection in the older roots or an increase in death rates. Alternatively, environmental conditions for the fungus can change and alter birth or death rates. We are only just beginning to understand the importance and changing functions of the different species of mycorrhizae. The models were really designed for studying interacting populations. The differing life spans of plants and fungi could make modeling a critical tool. All can be applied to given systems and used to better describe mycorrhizae.

Conclusion

Percent mycorrhizal infection is a useful variable that is often measurable in conditions where other parameters such as root length are difficult or impossible to ascertain. However, it should be borne in mind that this parameter is a composite of two important values: (1) presence of mycorrhizae and (2) relative balance of mycorrhizal fungi and root length. Modeling % infection using a logistic or any other single value perpetuates the false concept that a single function represents the amount of mycorrhizae. When two or more organisms live symbiotically, their population dynamics should be understood first separately and then with respect to each other. Modeling mycorrhizal activity by considering the involved organisms independently and then together holds the key to our understanding of this relationship.

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