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On the relation between arbuscular mycorrhizal colonization and plant 'benefit'

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A simple model is proposed which describes the relation between the extent of mycorrhizal colonization of a plant and the 'benefit' (positive or negative) derived by that plant. 'Benefit' is defined as the percent change in a plant performance parameter of a mycorrhizal individual relative to the mean of a number of mycorrhizal plants, grown in identical conditions. The model predicts a general curvilinear relation between colonization density and benefit, where benefit is maximised at some value of colonization. It is based on the fact that the relation of plant P uptake to mycorrhizal colonization is often non-linear. Four examples of empirical data which provide a good fit to the model, with third order polynomial regression are given. It is suggested that if the curvilinear relation is of general occurrence then it can provide an explanation for many of the apparently anomalous results seen in the mycorrhizal literature.

Arbuscular mycorrhizal (AM) fungi are ubiquitous in all ecosystems of the world, where they are known to form associations with about 70% of the world's vascular plant flora (Brundrett 1991). There is an extensive literature which documents the beneficial effects that these fungi can have on plants. Such effects include simple plant growth promotion through increased nutrient access and/or competitive ability (examples in Smith and Read 1997), enhanced drought resistance (e.g. Ruiz Lozano and Azcon 1995) and enhanced resistance to insect herbivores (e.g. Gange and Bower 1997) or plant pathogens (e.g. West 1997).

Scattered throughout the literature are examples of situations in which AM fungi have resulted in negative effects on plant growth. The elegant experiments of Francis and Read (1995) have shown that a wide variety of plant species can be adversely affected by AM colonization, when grown in conditions simulating natural communities. These authors have proposed a continuum of plant responses to AM colonization, from the (traditionally accepted) mutualistic through to antagonistic. The latter theme has also been taken up by Johnson et al. (1997) in their comprehensive and thought-provoking review. These authors discuss instances in which mycorrhizas have been shown to be detrimental to plants and present a number of reasons as to why these events may happen.

However, of less frequent occurrence in the literature is the situation in which *nothing happens* in terms of the plant factors outlined above, when AM colonization is experimentally increased or decreased. We wonder if this is simply a feature of the literature, in that null results are less often published, and suspect that it is a common occurrence. Certainly, from our personal experience, one can easily change AM colonization levels of a plant quite dramatically, and yet fail to measure any significant change in the plant. The situation of commensalism (gain for the fungus, while the plant neither loses nor gains) is described by both Francis and Read (1995) and Johnson et al. (1997), but not discussed at length.

We are therefore left with a situation in which AM fungi can clearly elicit a continuum of responses in a host plant, from positive, through null to negative. Indeed, in any particular host plant - fungus combination, the response can move along this continuum. For example, there is plenty of evidence to show that plant responses to AM colonization are positive when P is limiting, but negative when it is in abundance (Smith and Read

1997). In order to more fully understand the nature of the mycorrhizal symbiosis, Johnson et al. (1997) suggest that we need to explore predictive models of mycorrhizal functioning, which will need to incorporate variables and parameters that account for the responses of plants to the fungi. In this paper, we do not claim to have built such a comprehensive model, and our aim is certainly not to present a unifying theory of mycorrhizal functioning. However, we suggest that in order to start somewhere, we need to examine the responses of individual plants to AM colonization, over a range of colonization densities. As Johnson et al. (1997) point out, the degree of AM colonization of a plant is very important, and thus examining responses at different levels of fungal abundance may prove instructive.

Problems of measurement

There are two ways of manipulating AM colonization density of a plant, either by increasing the level relative to a control by adding inoculum, or by taking a naturally-occurring level and reducing it with a fungicide. The former approach is most often used in laboratory trials, while the latter is most often employed in field trials, although Jakobsen (1994) recommends the use of inoculation in the field also. This difference in method alone may be one important reason why the results of these trials do not always match up, as colonization densities may not be comparable and fungicides can have non-target effects on other organisms, such as plants and insects (Gange and Bower 1997). Furthermore, at least some of the extensive array of biotic and abiotic factors which may affect the functioning of the mycorrhiza in field situations may be reasonably well controlled or even eliminated in the laboratory.

In addition to the problems associated with the methods for conducting the experiment, a second difficulty in formulating any model is to consider what plant parameters to measure. A host of measurements have been used in the past, encompassing plant morphology, allometry, phenology and chemistry (Johnson et al. 1997). There is clearly a need to implement a standardised measurement of 'benefit' to the plant in forming the mycorrhizal association, which we attempt to define below.

A further problem with measurement concerns the manner in which colonization of the plant by the fungus is recorded. The vast majority of studies employ staining of the root, usually following the methods of Phillips and Hayman (1970) or Koske and Gemma (1989). However, it is a fact that different visualization methods produce very different results, even on the same plant (Gange et al. 1999). Various structures of the mycorrhiza may be recorded; some authors record arbuscular, vesicular and hyphal colonization separately, others do not. Given that the arbuscule is the definitive, functioning unit of the mycorrhiza, we suggest that measurement of arbuscular colonization levels only, is the best way of ensuring comparable data sets (c.f. Gange et al. 1999).

A striking feature of the literature is that in the vast majority of experiments, authors have made comparisons between means of 'mycorrhizal' and 'non-mycorrhizal' or 'reduced mycorrhizal' plants. Such an approach is quite valid, given the performance of a suitable comparison of means test; it is a logical step to portray means of treatments and to quantify any differences between them. Unfortunately, such portrayal can hide some very interesting data and rarely has the response of a plant been depicted with a scatter plot in which the *x*-axis represents amount of root colonization, and the *y*-axis the plant parameter measured, with individual plants as the data points. It is this latter approach upon which our simple model is based.

Model parameters and assumptions

We define plant 'benefit' in the model as the percentage change in a vegetative or reproductive parameter of a mycorrhizal plant relative to a mean value for plants without AM colonization. Suitable plant performance measurements could be dry biomass, leaf number, height, flower number, fruit or seed yield etc. Thus,

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where *m* is the performance parameter of an individual mycorrhizal plant and *n* the mean of that performance parameter of mycorrhizal-free plants, grown in the same experimental conditions. We have taken this general approach so that the wide variety of plant performance parameters affected by mycorrhizal fungi can be encompassed. The

ultimate measure of plant 'benefit' must be linked to reproductive success, i.e. genetic fitness. Parameters such as seed number, size, and viability and seedling success could easily be used in this equation. However, responses in plant reproductive parameters to AM fungi are less often examined than vegetative characters (but see Koide XX)

Our measurement of AM colonization of the root is percent root length colonised by arbuscules only, for the reasons given above.

Given the above, we assume that 'benefit' is zero when AM colonization is zero. In other words, there cannot be any direct influence of the mycorrhiza on a plant which has not been colonized.

A simple model

Our aim in developing this simple model is to provide a testable hypothesis concerning the degree of AM colonization of a plant and the effect which this has on that plant. Furthermore, we hope to encourage testing of this hypothesis by providing a modelling framework which can be subject to experimental examination.

Our hypothesis is that

There is a curvilinear relation between AM colonization density and plant 'benefit'.

This is similar to a model proposed by Fitter and Sanders (1992), describing a cost benefit analysis of grazing on the mycorrhiza by soil arthropods. However, our model is fundamentally different as it considers individual plant responses and encompasses any plant response parameter in a standardised manner. Similar to the reasoning by Fitter and Sanders (1992), we suggest that there is an optimum density of mycorrhizal colonization for a plant. From zero up to this optimum, increased P uptake via the mycorrhiza leads to increased growth, through nutrient uptake and photosynthesis. Meanwhile, colonization above the optimum represents an increasing carbon drain by the mycorrhiza which may counteract the effect on carbon fixation. There are many examples of plant growth reductions caused by high levels of AM fungal colonization being attributed to the carbon demand of the mycorrhiza (e.g. Buwalda and Goh 1982, Peng et al. 1993, Marschner and

Crowley 1996, Graham and Eissenstat 1998). However, loss of photosynthate to the mycorrhiza is not the only reason why negative effects on plant growth may be seen. Mycorrhizas may compete with plants for nutrients, immobilize N, affect root exudation and the rhizosphere microflora, all of which could lead to negative effects at high colonization densities (Bethlenfalvay et al 1982, Johnson et al. 1997, Marschner and Crowley 1996). Our suggested relation is non-linear, based on the fact that previous authors have recorded curvilinear relations between plant P uptake and grazing pressure on the mycorrhizal mycelium (Finlay 1985, Harris and Boerner 1990). Variation in the extent of grazing on the mycorrhiza should be equivalent to a variation in mycorrhizal colonization density.

Therefore, we suggest that over a range of colonization densities, from zero to 100%, 'benefit' will increase, reach a plateau and then decline. It can become negative. A simple graphical depiction of this statement is given in Fig. 1a. This line represents the 'benefit' exhibited by individuals of a plant species, to different levels of AM colonization by one species of mycorrhizal fungus, under highly controlled conditions, i.e. each plant receives the same amount of water, light, nutrients etc. In truth, there is more likely to be a family of curves, such as shown in Fig. 1b. For example, two factors known to reduce AM colonization are high soil P and low irradiant light (Son and Smith 1988). In this case, the range of curves from A-D could represent mycorrhizal colonization and corresponding plant 'benefit' at progressively increasing levels of soil P and/or decreasing levels of irradiant light. In curve A, 'benefit' is seen over a wide range of colonization densities. However, as P level increases or light decreases, so the maximum value of 'benefit' is achieved at a lower colonization rate, and the number of instances of the mycorrhiza being antagonistic (negative 'benefit') increases.

The family of curves could also represent different AM species being used in the experiment. Species-specific responses of plants to different fungi have been reported (e.g. Sanders et al. 1977, Streitwolf-Engel et al. 1997). Thus, curve A could represent host plant responses to a fungus which is beneficial at virtually any colonization density, while curve D is a fungus which is antagonistic at virtually any colonization density. An example of the latter situation would be *Glomus macrocarpum* on tobacco (*Nicotiana tabacum*) (Modjo and Hendrix 1986).

One may also envisage the curves as the responses of different plants to the same fungus. Thus, for example, curves A and B may represent the response of *Medicago sativa* to *G. macrocarpum*, which elicits a positive growth response in this plant (Srivastava and Mukerji 1995). However, curve D may represent the response of tobacco to this fungus, where *G. macrocarpum* is mainly antagonistic (An et al. 1993). As species-specific responses of plants to AM fungi are clearly important in community ecology (van der Heijden et al. 1998), modelling of these responses in relation to colonization densities may be particularly instructive.

Some predictions

The model may explain some apparently anomalous results that can be obtained from manipulative experiments. Let us consider the response curve in Fig. 2. In this situation, the maximum 'benefit' to the plant occurs when about 37% of the root system has been colonised. If there is natural colonization of about 60% (point A), and this is successfully reduced by a fungicide to, say 10% (point B), then there may be no actual difference in growth response of the plant. It could therefore be assumed that the mycorrhizal fungi had no effect on plant growth. Alternatively, if we are to the left of the curve peak (point C) and successfully reduce colonization to point D, then a reduction in 'benefit' is observed and the mycorrhiza in this case could be considered 'beneficial'. Meanwhile, if we are at point A and reduce colonization to that of point C, plant 'benefit' actually increases, such that the mycorrhiza could arguably be considered antagonistic. The actual shape of the curve will be critical in predicting these responses, for example, there may be a much sharper peak, or alternatively it may be a plateau, with a flat top to the curve.

Empirical evidence

Example 1

Clapperton and Reid (1992) present graphical data to show the relation between colonization density (percent root length colonised, hyphae and arbuscules) and plant dry weight in *Phleum pratense* and *Agropyron trachycaulum*. In this case, the data points

were means of plant and fungal parameters at different soil dilutions, in a dilution series. However, in all cases presented, the relation was curvilinear, modelled by a third-order regression.

Example 2

McGonigle (1988) presented a numerical analysis of field trials with AM fungi. In this paper, an analysis was conducted between standardised AM colonization increase (*x*-axis) and the corresponding standardised change in yield (*y*-axis) for 78 trials. A linear relation was sought but found to be non-significant ($F_{1,76} = 2.3$, $P > 0.05$). If, however, one examines this relation with polynomial regression, then a reasonable fit is obtained with a third order polynomial ($F_{3,75} = 11.1$, $P < 0.001$, $r^2 = 30.8\%$) or a second ($F_{2,76} = 14.6$, $P < 0.001$, $r^2 = 27.8\%$.).

Example 3

The data comes from a recent experiment described in Gange and Nice (1997). In that study, plants of *Cirsium arvense* were grown in pots, with fungicide being added to reduce AM colonization as one treatment. Changes in plant growth are likely to have been a direct result of AM reduction, as no plant pathogenic fungi were detected in the roots in this experiment (Gange and Nice 1997). Furthermore, application of fungicide had no effect on soil N levels (mean of control pots = XX±, mean of fungicide pots = XX±).

The 'benefit' data plotted in Fig. 3 were derived from the equation given above, using dry weight as the performance parameter. The values of 'benefit' so obtained were normally distributed. Colonization density on the *x*-axis is plotted as percent root length colonised (arbuscules only). The fitted line is a third order polynomial ($F_{3,57} = 35.6$, $P < 0.001$, $r^2 = 65.2\%$) but a second order polynomial provides an equally good fit ($F_{2,58} = 54.3$, $P < 0.001$, $r^2 = 65.2\%$).

Example 4

The data comes from a recent experiment (Gange unpublished) in which *Conyza canadensis* was grown singly in pots of John Innes number 2 compost and varying amounts of inoculum of the fungus *Glomus intraradices* added as a mixture of spores and hyphae in an inert clay carrier. Control plants were given irradiated inoculum. 'Benefit' was calculated in the same manner as above and the values obtained were normally distributed. Colonization density is also expressed as %RLC (arbuscules only) and the data are plotted in Fig. 4. The fitted line is a third order polynomial ($F_{3,97} = 57.4$, $P < 0.001$, $r^2 = 63.9\%$).

Model criticism

In our model, and in the fitted lines, we have forced the line through the origin (i.e. no constant). This clearly affects the significance of the regression, but we believe this is a valid approach. The *y*-axis in our model represents a *degree of benefit*, which is a relative measure, not an absolute one. Hence 0,0 is a valid data point because, given our definition, a plant without the mycorrhiza cannot receive any 'benefit'.

In our experiments, we did not account for genotypic variation. Such natural variation will cause a scatter of points around the line, and we suggest that a tighter fit may be obtained using plant material which is genetically identical.

We have found in our studies that the best fit to the data sets was produced by a third order polynomial. Clapperton and Reid (1992) fitted third order regressions to their data and such a regression allows for the curve to asymptote at a negative value, implying that beyond a certain colonization level, no greater ill-effects are seen. This may be a more biologically realistic scenario than the increasing negative effect implied by a second order polynomial.

In our studies, we have examined the responses to colonization using individual plants as points in the regressions. Ideally, one should grow many plants and obtain mean values for 'benefit' and colonization density at as many values of colonization as possible. This is the approach taken by Clapperton and Reid (1992). The problem here is that this involves a tremendous amount of work to obtain a large number of data points. For example, Clapperton and Reid (1992) have only six data points on their graphs.

One of the most important aspects of any mycorrhizal trial is the time over which it occurs. There are plenty of examples in which antagonistic effects of the fungi are seen in the early stages of the growth of annual plants, which disappear as the plants become mature (Johnson et al. 1997). Furthermore, mycorrhizas may have negative or positive effects on growth of perennial plants, depending on the time of year (Lapointe and Molard 1997). Therefore, an obvious amendment to our model would be to add a time axis, in order to obtain a response surface.

Conclusions

We hope that the simple model presented here will stimulate researchers to examine the relations between the degree of colonization of roots by AM fungi and the responses of individual plants. In this way, we may be able to account for some of the observed differences in plant responses to mycorrhizal inoculation or chemical reduction. We may then be able to progress towards developing a full model of mycorrhizal functioning as proposed by Johnson et al. (1997), that is applicable to both field and laboratory.

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Figure legends

Fig. 1a. The proposed curvilinear relation between mycorrhizal colonization density and plant 'benefit'. The *y*-axis is not numbered, as this may be to any scale, depending on the study system. The model predicts that over a range of colonization densities, there will be a positive effect of the mycorrhiza on plant performance, but only up to a point; after this 'benefit' declines and can become negative if colonization is too high.

b. It is likely that a family of curves exist, each one may represent a different scenario for a particular plant or fungus combination. The *y*-axis is again numberless but may be to any scale. See text for a full explanation of Figure.

Fig. 2. Some predictions which arise from the model. A reduction in mycorrhizal colonization may result in increased (A $\rightarrow$ C), decreased (C $\rightarrow$ D) or no effect on (A $\rightarrow$ B) plant performance. The numerical scale is purely for ease of explanation.

Fig. 3. An empirical test of the model using *Cirsium arvense*, grown in pots in the field. Fitted line: $y = 5.9x - 0.1x^2 + 0.0001x^3$.

Fig. 4. An empirical test of the model, using *Conyza canadensis* inoculated with *Glomus intraradices*, and grown in a constant environment of 20°C and 16:8 L:D. Fitted line: $y = 69.8x - 6.34x^2 + 0.13x^3$.

Fig.1

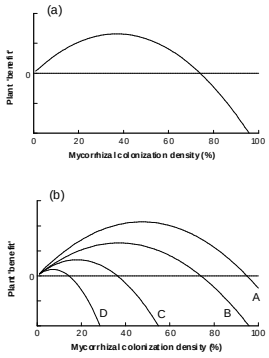

Fig 2

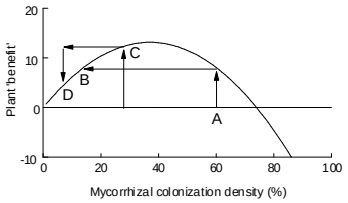

Fig. 3

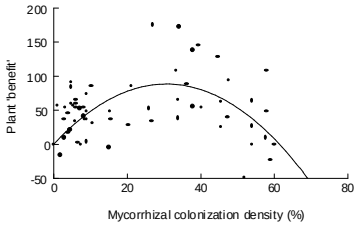

Fig. 4