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John N. Klironomos · Miranda M. Hart

Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum

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Abstract Arbuscular mycorrhizal fungi (AMF) form a number of different infective propagules that are used to form new mycorrhizal associations. These are spores, extraradical hyphae and infected roots. However, not all fungi are equally capable of colonizing roots with all of the above-mentioned propagules and there is conflicting evidence of major differences in colonization strategy between members of the Glomineae and Gigasporineae. In this study, we tested the abilities of eight fungal species from four different genera to colonize roots using three different types of inoculum. *Glomus* and *Acaulospora* isolates colonized from all inoculum types, whereas *Gigaspora* and *Scutellospora* isolates colonized mainly from spores and to a limited degree from root fragments. Extraradical hyphae were not suitable propagules for the species of *Gigaspora* and *Scutellospora* tested. This indicates that AMF have different colonization strategies and that this is largely differentiated at the suborder level. It is unclear why there is such a difference among the fungi in inoculum types. Future research should examine differences in the anatomy and physiology to discern a mechanism for such differences in life-history strategies.

Keywords Arbuscular mycorrhiza · Inoculum type · Colonization strategy

Introduction

Arbuscular mycorrhizal fungi (AMF) can use a number of different types of propagules to colonize new roots. Typically, these propagules are considered to be components of the extraradical phase of AMF. The extraradical phase comprises spores and a mycelium that includes the absorptive hyphal network and runner hyphae (Friese and Allen 1991). The former is responsible primarily for nutrient uptake from the soil, but the latter grow along or among root segments and form new infection units. Spores develop from the extraradical mycelium and are also highly infective. Germ tubes grow from the spores, extend for several centimeters in the direction of active roots and ultimately develop a primary infection.

Components of the intraradical phase can also be infective. Inside roots, AMF form several structures, mainly arbuscules, vesicles, coils and unspecialized hyphae. Of these, vesicles have been shown to be particularly infective. As a result, living and dead root segments can also be a source of inoculum for newly developing roots (Tommerup 1984).

Although a number of different propagule types exist, they may not be equally effective at producing new infection units. For example, it would be expected in undisturbed soil that new infection units arise primarily from extraradical hyphae and that spores are less important. This is mainly because it would take longer for spores to germinate and make contact with roots as opposed to runner hyphae infecting from a well-developed extraradical mycelium. Soil that is disturbed, however, might result in damaged hyphae that are non-infective (Jasper et al. 1989). In such a case, spores may be the preferred method of propagation.

There is some indication that the relative contribution of each source of inoculum differs among taxa of AMF (INVAM 1993). In particular, it seems that the hyphal component may be more important for species of *Glomus*, whereas the spore component may be more important for species in other genera. This is supported by several studies. Abbott et al. (1994) showed that mycorrhizal root pieces were effective propagules for *Glomus* and *Acaulospora* isolates but not for *Scutellospora* isolates. Biermann and Linderman (1983) reported a similar result. They examined the role of root fragments as propagules and found high infectivity from those containing *Glomus* and *Acaulospora* species, but none from root fragments containing *Gigaspora* species. They attributed this difference to the presence of vesicles. In the same study, they extracted vesicles from the root

J.N. Klironomos (✉) · M.M. Hart
Department of Botany, University of Guelph, Guelph, Ontario, Canada N1G 2W1
e-mail: jklirono@uoguelph.ca
Tel.: +1-519-8244120 ext. 6007, Fax: +1-519-7671991

fragments and found the vesicles to be infective but not the remaining root/hypha debris. Most recently, Brundrett et al. (1999) tested the success of establishing a diversity of AMF into pot culture from the field using different sources of inoculum. They found that field-collected spores were useful for establishing most species of *Acaulospora*, *Gigaspora*, and *Scutellospora*, whereas little success was found with root fragments. On the other hand, they found that *Glomus* species were rarely recovered from field-collected spores but were dominant when using root fragments or intact soil cores.

Although most studies suggest dependence on spores for successful colonization by members of *Gigaspora* and *Scutellospora*, other studies contradict this trend. Tommerup and Abbott (1981) studied the infectivity potential of 6-month-old, dried root fragments containing one of two *Glomus* species, one species of *Acaulospora* and one species of *Scutellospora*. They found that hyphae emerged from within the dead root fragments for the *Glomus* species and the species of *Scutellospora* and created new infections in the presence of a viable host plant. Also, Braunberger et al. (1996) found that one species of *Scutellospora* was able to colonize new roots from a dried root inoculum.

Overall, previously published studies point to different colonization strategies among species of AMF. However, it is still not clear whether the major differences occur at higher taxonomic levels (genus-suborder), in part because previous studies used different experimental approaches and comparisons among studies are confounded by different environmental conditions. The objective of this present study was to test for differences in colonization strategy among different AMF taxa under controlled growth conditions. We examined the ability of eight AMF from four different genera to successfully colonize roots of *Allium porrum* using different types of propagules (spores, colonized root fragments with associated hyphae, and extraradical hyphae only). We hypothesized that species of *Gigaspora* and *Scutellospora* would achieve more successful colonization of roots from spores, with limited ability to colonize from root fragments and extraradical hyphae. In contrast, we hypothesized that species of *Glomus* and *Acaulospora* would have equal success colonizing plant roots from all inoculum types tested.

Materials and methods

Fungi

Eight AMF were used, all isolated in 1997 from the Long-Term Mycorrhiza Research Site, University of Guelph, Canada (Klironomos 2000). These fungi were maintained in pot culture under greenhouse conditions until the start of the experiment. Pot cultures comprised *A. porrum* seedlings growing in 6-inch pots containing Turface. Each pot contained a single AMF isolate. Plants were watered with deionized water and fertilized with Long Ashton nutrient solution as needed. The fungi chosen were four representatives of each of the two suborders within the Order Glomales, as listed below:

1. Suborder Glomineae: Family Glomaceae

- *Glomus intraradices* Schenck & Smith
- *Glomus etunicatum* Becker & Gerdemann

2. Suborder Glomineae: Family Acaulosporaceae

- *Acaulospora spinosa* Walker & Trappe
- *Acaulospora morrowiae* Spain & Schenck

3. Suborder Gigasporineae: Family Gigasporaceae

- *Gigaspora gigantea* (Nicol. & Gerd.) Gerdemann & Trappe
- *Gigaspora margarita* Becker & Hall
- *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders
- *Scutellospora heterogama* (Nicol. & Gerdemann) Walker & Sanders

Experimental set-up

We set up 30 experimental units for each of the eight fungal species. The 30 experimental units were further divided into three inoculum-type treatments: (i) spores, (ii) washed roots with small pieces of extraradical hyphae and (iii) extraradical hyphae only. Ten additional experimental units were prepared without AMF inoculum. Each experimental unit consisted of an 8-inch pot, containing a 1:1 ratio of silica sand and Pro-Mix BX (Premier Horticulture Inc.). The two substrates were mixed together and then autoclaved. Each pot was then 3/4 filled with the potting mix. On top of this we added one of the three inoculum types. For the spore treatment, spores were extracted from the previously established pot cultures (6 weeks old) using a wet sieving technique (Klironomos et al. 1993). One hundred healthy spores from one of the eight fungi were added to each pot. Previous tests showed that spores of all species tested had high percent germination (>80%). For the washed-root treatment, roots from 6-week-old pot cultures were washed free of their growing medium using tap water. Subsamples of roots were inspected at ×40 magnification and were found to be free of AMF spores; however, small pieces of extraradical hyphae were still connected. Staining subsamples showed that percent colonization of roots was greater than 50% for all species tested. One gram of washed roots containing one of the eight fungi was added to each pot. For the extraradical hypha treatment, fungal hyphae were extracted from 6-week-old pot cultures using the method described by Miller et al. (1995). One hundred hyphal fragments (>1 mm long) were transferred to each experimental unit. Preliminary analysis with a Europium-based differential fluorescent stain (Morris et al. 1997) indicated that more than 50% of hyphal fragments were viable after extraction for all AMF taxa. After the appropriate inoculum type was added, each pot was further filled with the sterile potting mix. To each pot, we then added 10 seeds of *A. porrum*. Since some seeds did not germinate, we removed some seedlings at week 2 so that five plants remained in each pot.

Pots were placed on a greenhouse bench using a completely randomized design. Plants were watered as needed and fertilized on a weekly basis with Long Ashton nutrient solution. Roots from all pots were harvested after 2 months. Roots were removed, washed free of potting mix and stained with Chlorazol Black E (Brundrett et al. 1984). Percent colonization by AMF was determined using the magnified intersections method (McGonigle et al. 1990). Percent colonization in this study refers to percent colonization by arbuscules or vesicles.

Statistical analysis

The dependent variable in this study was percent colonization by arbuscules or vesicles. A 3×8 factorial analysis of variance (ANOVA) was used to test for statistically significant differences among the three types of inoculum and eight AMF species. Each

AMF species was further analyzed for differences in percent colonization among inoculum types using one-way ANOVA, followed by a Tukey post-hoc test.

Results and discussion

We found clear evidence of differences among AMF species in their ability to colonize roots using different inoculum types. Using a factorial ANOVA, we detected a significant AMF species main effect (P=0.005), a significant inoculum type main effect (P=0.0001) and an AMF species × inoculum type interaction (P=0.0001). The species of *Glomus* and *Acaulospora* were equally able to colonize roots of *A. porrum* from spores, root fragments, and hyphae (Fig. 1). There was no significant difference in the resulting percent colonization among different inoculum types for these species. In contrast, root colonization by *Gigaspora* and *Scutellospora* was only successful when spores were used, with the exception of a low percent colonization (6%) by *Scutellospora heterogama* using root fragments. No colonization with arbuscules or vesicles was found in non-inoculated control plants. These findings support previous results showing that *Glomus* and *Acaulospora* isolates were able to use root fragments and hyphae as significant propagules (Biermann and Linderman 1983; INVAM 1993; Abbott et al. 1994; Brundrett et al. 1999).

Root fragments were infective in species of *Glomus* and *Acaulospora*; however, it is not possible to determine which fungal component within the root is infective, the intra- or extraradical hyphae, vesicles, or all of them. A study by Biermann and Linderman (1983) suggested that only vesicles are infective. They isolated vesicles from *Glomus fasciculatum* and *Glomus mosseae* and found them to be infective, but the remaining hyphal fraction within the roots was not. However, it is possible that the intraradical hyphal fraction was infective but damaged in the extraction process. Two other studies provide evidence that the intraradical hyphal network is likely to be infective (Tommerup and Abbott 1981; Braunberger et al. 1996). They both illustrate that AMF from root fragments of *Scutellospora calospora* can successfully colonize new roots. This fungus does not produce vesicles, so the infection must have arisen from intraradical hyphae or regrown extraradical hyphae mycelium associated with the root fragments. In the present study, we also showed that AMF which lack vesicles can successfully colonize new roots from root fragments, albeit with limited success. In addition, it is not known whether other environmental factors in the soil influence the infectivity of fungi associated with root fragments. For example, AMF within root fragments may be more effective propagules after significant decomposition of the root fragments.

The present results indicate that extraradical hyphae are infective in species of the Glomineae but not in species of the Gigasporineae. If this trend is consistent with other species that have not been tested here, then it suggests that the extraradical architecture of AMF differs

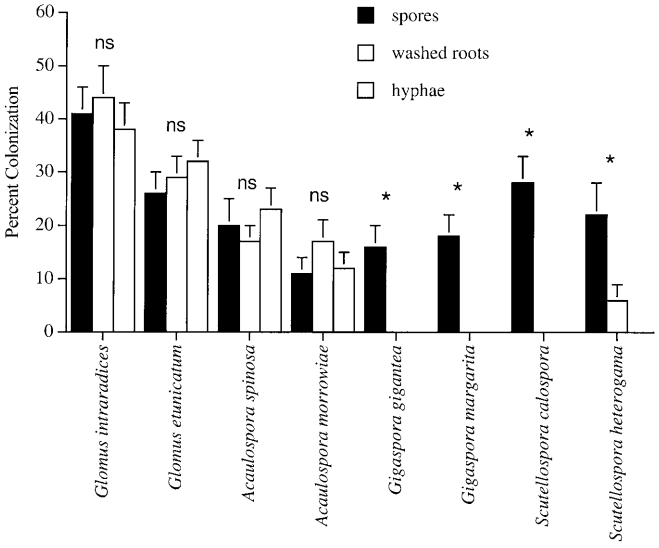

Fig. 1 The influence of arbuscular mycorrhizal fungal species and inoculum type on percent colonization by arbuscules and vesicles. Data were subjected to one-way ANOVA, n=10 (*P<0.05; *ns* P>0.05). All non-inoculated control plants were not colonized

significantly between these two suborders. While both groups may produce absorptive hyphal networks, members of the Gigasporineae may lack runner hyphae (Friese and Allen 1991). Alternatively, our results may also have been due to differences in the disturbance tolerance between the two groups of fungi, rather than differences in life strategy. Members of the Gigasporineae may simply be more sensitive to disturbance than species of the Glomineae. The hyphal fragments used in this study were severed pieces of the extraradical mycelium. Based on preliminary tests, hyphal fragments from the various AMF taxa were equally viable after disturbance. Nevertheless, whereas the intact mycelium may have enough energy supply to be infective, the severed hyphal fragments may have lost this ability. AMF hyphae are coenocytic and severing them results in the loss of some cytoplasm. In response, some AMF form cross walls near such injuries, thus allowing their cytoplasm to be redirected to intact areas of the mycelium (personal communication). From our observations, we have noticed the formation of such cross walls in species of *Glomus*, but far fewer in species of *Gigaspora* and *Scutellospora*. It is likely that members of the Gigasporineae have developed other strategies to protect their extraradical mycelium from being severed in nature. For example, their hyphae and cell walls are typically coarser than those of *Glomus* or saprobic fungi, making it more difficult for soil animals to graze on them (Klironomos et al. 1999). However, such a strategy would not be of benefit with the extraction methods used in this study and, thus, the present results or those from any other experiment that uses hyphal fragments may be confounded by hyphal disturbance.

Our results do not fully support the findings of Tommerup and Abbott (1981) and Braunberger et al.

(1996), who found that an isolate of *S. calospora* was able to form an infection from a mycorrhizal root fragment. Our isolate of *S. calospora* did not successfully infect new roots from pre-infected roots as inoculum. This indicates that high functional variability may exist within individual species of AMF. We did find that another AMF isolate from the Gigasporineae (*S. heterogama*) was capable of colonizing new roots from pre-infected roots; however, this resulted in a low level of infection. It may be that there is variation within groups of AMF in how well they are able to recover from disturbance and thus use root fragments as propagules. However, it is not possible to provide an adequate mechanistic explanation for the present results, since differences in the anatomy/physiology of AMF species are not well understood. Nevertheless, our results indicate the existence of different life-history strategies among AMF in terms of colonization and that these differences are taxonomically based. The consequence of this for the culturing of AMF species in pot cultures is significant. The present results suggest that higher success in culturing *Gigaspora* and *Scutellospora* species can be achieved using spores. *Glomus* and *Acaulospora* species can be cultured using any combination of spores, hyphae and roots.

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