

Recognition and communication events between arbuscular mycorrhizal fungi and host roots

G. Nagahashi, D. Douds, Jr. and G. Bécard*

U. S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038 and
Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse, France

ABSTRACT

At least six steps involving recognition, signaling, or communication between host root and arbuscular mycorrhizal (AM) fungi appear to be necessary for the fungus to complete its life cycle. Recent developments in *in vitro* culture technology, including the use of germinated aseptically fungal spores, roots grown in liquid culture, dual culture of a host root and single spore on a solid support medium, and the split plate technology, have provided the means to study specific steps in detail. Although *in vitro* culture is greatly simplified over the root-soil environment, it has provided a way to study early recognition events between a single germinated fungal spore and a specific host root without complication from other rhizosphere components.

INTRODUCTION

The signaling processes between host root and arbuscular mycorrhizal (AM) fungi before and after colonization are poorly understood. The life cycle of the obligately symbiotic fungus entails germination of a spore, stimulation of fungal growth and branching by the presence of a host root, appressorium formation, penetration and intercellular spread, arbuscule formation, extraradical hyphal growth, and finally, sporulation. A major challenge facing researchers is to culture

these fungi in the absence of a host root. The events which lead to morphogenetic changes of the fungus must be elucidated in order to complete this difficult task. Current knowledge of one-way or two-way signaling events between AM fungus and host before and during colonization of the root will be discussed.

AM FUNGUS LIFE CYCLE AND SIGNALING PROCESSES

Germination of spores

Spore germination is a major step in the life cycle of an AM fungus and is influenced by spore dormancy, environmental conditions (pH, temperature, water potential, CO₂ concentration), and soil microbial activity. There is some evidence that plant products in root exudates may play a role in germination [1], but this is not a universal phenomenon. Plant signals or compounds may not be necessary for germination and, in fact, spore germination and hyphal growth have been observed routinely with the appropriate environmental conditions [1, 2, 3] in the absence of a host root.

First signaling event between host root and AM fungus

As AM fungal germ tubes grow through the soil, they must find a suitable host root in order for

the fungus to complete its life cycle. There is some evidence that roots give off a volatile signal that stimulates directional growth of the germ tube of *Gigaspora gigantea* toward the root [4]. Treatment with KMnO_4 to oxidize volatile organics and KOH to trap CO_2 reduced or eliminated the response [5]. A synergistic response between CO_2 and root exudates also has been reported [6]. Hyphal growth was slightly stimulated by root volatiles and was not stimulated by root exudates alone, but in the presence of CO_2 , a considerable increase in hyphal growth was observed [6].

Chemical compounds are mainly released to the soil via the root exudation process and the exudate can contain water soluble and volatile components [5]. There is no evidence that water soluble exudates attract germ tubes of AM fungi. It is generally believed that these compounds are not chemotropic but they induce a response by the fungus which increases the probability of contact with the roots.

The most typical reported response of AM fungi to host root exudates is extensive hyphal growth [1 and references therein]. Roots of non-host species neither enhance nor inhibit hyphal growth of AM fungi, indicating that these plants are lacking the necessary stimulatory factors [1]. Some authors have reported that host root exudates not only stimulate hyphal growth but also promote a morphogenetic effect (increased hyphal branching) in AM fungi [7, 8]. It is well documented that hyphal branching increases in the presence of host roots or their exudates and in particular, hyphal tips branch profusely as they grow within a few mm of a host root (see 1 for a recent review). The branching was readily seen when roots and germinated AM fungal spores were separated by a membrane which allowed soluble compounds to pass through the membrane and promote hyphal branching over areas in closest proximity to the roots.

Whether two separate types of signals, one for elongation and one for branching, are actually present or whether these morphological responses are simply dose dependent has not yet been determined. Our work with TLC and HPLC separation of exudate components support the latter. Six active fractions were separated by TLC and each active fraction caused fewer but longer

branches when tested in a diluted form and extensive branching when they were tested after a concentration step (Nagahashi and Douds, unpublished data). These results also indicate that there are multiple branching signals found in the exudate of a host root.

Another significant observation indicated that the exudates from roots grown with adequate phosphorus induced significantly less branching of hyphae than exudates from P-stressed roots [9]. This implied that more signal was generated by P-stressed roots and was consistent with the observation that P-stressed plants had a greater percentage of root length colonized by *G. margarita* and greater sporulation than plants grown with P [10 and references therein].

Recently, the type of hyphal branching normally induced by the presence of a host root was duplicated in an *in vitro* culture system using only concentrated host exudate and germinated AM fungal spores [11]. This was the first time the hyphal branching was achieved without a host root and was successful because of the development of a new method for monitoring branching signals generated by host roots.

The method involves the use of single aseptic AM fungus spores which are transferred to solid medium upon germination. After 3-5 days of hyphal growth, concentrated exudate is injected with a tuberculin syringe into a small hole in the support medium 2-3 mm in front of a growing hyphal tip. The plates are incubated at 32 °C in a 2% CO_2 atmosphere and hyphal branching is monitored periodically. The branching response can be seen within 4 hr but hyphae are typically observed 15 hr after injection for convenience. This method has been shown to work for *Gigaspora gigantea*, *Gigaspora margarita* and for *Glomus intraradices*. The exudate used to test these species was obtained from transformed carrot roots grown in liquid culture for two weeks and then transferred to liquid medium minus P for an additional week. The liquid culture medium was then filtered to remove roots and sloughed root caps and the exudate was concentrated on a SEPAK C18 cartridge. The cartridge then was eluted with 50% methanol and this methanol wash was discarded. A subsequent elution with 70% methanol provided the

source of partially purified branching signals. The compounds inducing fungal branching were not volatile and were initially found in the aqueous exudate.

Because concentrated exudates from transformed roots of carrot, non-transformed cultured tomato roots, and roots of corn seedlings all induce rapid branching in this method, the technique provides for a rapid and sensitive bioassay with a broad application. This bioassay can now be used to test any isolated fraction separated by TLC, HPLC, or column chromatography or any pure, commercially available, chemical compound for branching activity.

Although the assay is very sensitive, it is not quantitative. It cannot directly enumerate how much branching activity is present, only that it is present. This limitation to the assay has been partly reconciled by two types of experiments. The branching can be monitored at various concentrations of signal. Low dosage typically gives fewer branches (the branching is still considerably greater than the controls without exudate) but more elongated branches (Figure 1). Increasingly higher dosages (Figure 2) yield bushier, three dimensional-type branching; crinkled, curly type branching; and finally at a high dose, arbuscule-like branching. The arbuscule-like branches are very stunted, finger-like branches which resemble arbuscules in colonized host roots and occur within 15 hr in the presence of CO₂. This type of branching pattern has not been observed, until now, for fungal hyphae before colonization of a root. However, it has been reported for external hyphae (extraradical hyphae) after colonization of a host root [12, 13] and when external nutrients are modified to limit colonization of a host root [14]. These results could be interpreted to mean that a very high concentration or localization of signal is necessary to stimulate arbuscule-like branching prior to colonization and physiological levels of the signal will only produce this effect after colonization of the host root. The physiological significance of this type of branching has not been determined although experiments are under way to determine if they are sites of glucose or nutrient uptake.

The type of branching pattern and the degree of branching of hyphae exposed to moderately concentrated exudate are identical to that of a germinated AM fungal spore growing in dual culture with a Ri T-DNA transformed carrot root. The branches are fewer and more elongated when the hyphal tips are farther from the roots, but bushy branching occurs as the tips grow close to the surface of the root. Similarly, by injecting concentrated exudate and observing the branching pattern for several days (Figure 3), one notes that as the hyphal tips continue to grow farther away from the injection sites, there is a concomitant dilution of signal due to diffusion. After several days, the hyphal tips farthest from the injection site exhibit fewer but more elongated branches compared to those nearest the injection site. Although not strictly quantitative, the use of one of these approaches in the *in vitro* culture test system can provide a rapid and sensitive bioassay for monitoring the isolation and purification of branching signals. Also, this assay could conceivably be used to monitor sequential morphogenetic changes of AM fungi as different signals are applied.

Appressoria formation

Appressoria are infection structures that are flattened, elliptical hyphal tips which form when germinated endomycorrhizal spores come in contact with a host root. The appressoria are the first cell to cell contact event and their formation is considered to be the most important evidence indicating the successful recognition between a fungus and host [15]. It was reported that a thigmotropic stimulus could not trigger hyphal differentiation into infection structures [16]. This conclusion was based on an experiment with threads of silk, cotton, nylon, glass or polyamide of various diameters. It was unclear if the threads were packed close together or with considerable space between them. If the spacing was far apart, a groove between two adjacent threads would not be apparent. In any event, no appressoria were formed on the threads even when in the presence of host root exudate. Since most appressoria form in a groove between two adjacent epidermal cells [17], the thread density

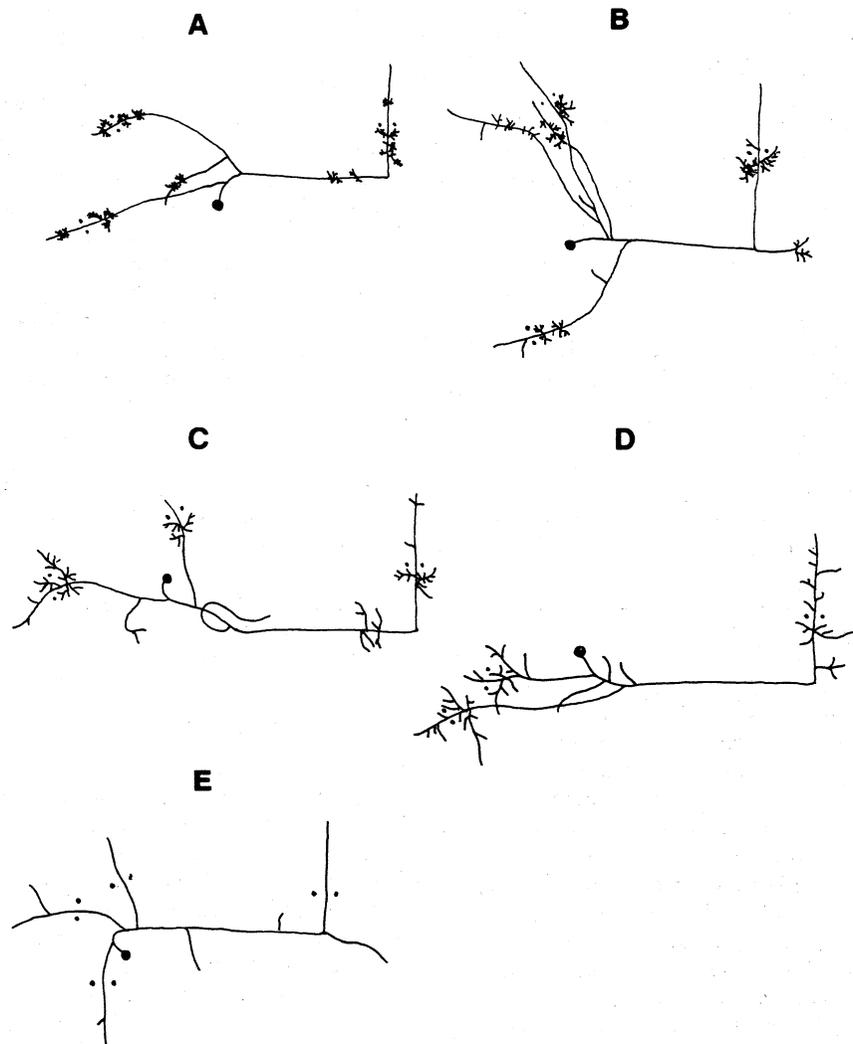


Figure 1. The dose dependent response of hyphal tips of *Gigaspora gigantea* to decreasing concentration of exudate which was collected from Ri T-DNA transformed carrot roots grown aseptically in liquid culture. The figures are actual tracings of the hyphal growth of pregerminated fungal spores grown on a solid support for 24 hr after the injection of exudate. Dots mark the sites of injection. A high dose of exudate stimulates arbuscular-type of branches which are stunted in growth. A low dose stimulates fewer branches but they are elongated. A. Concentrated exudate. B. Concentrated exudate diluted 1:100 with 70% ethanol. C. Concentrated exudate diluted 1:1000 with 70% ethanol. D. Concentrated exudate diluted 1:2000 with 70% ethanol. E. Control injected with 70% ethanol.

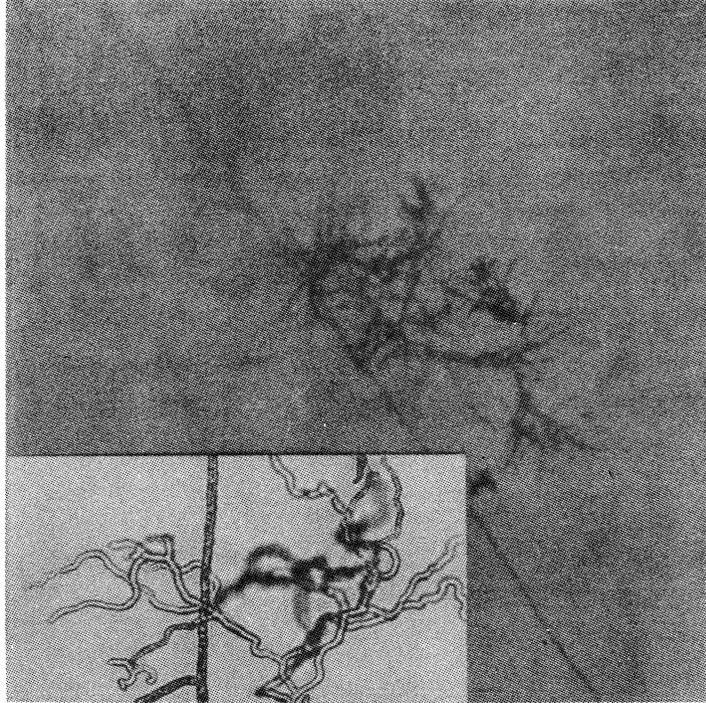


Figure 2. Micrographs of the hyphal branching patterns of *Gigaspora gigantea* in the presence of high concentration of semipurified branching signals from carrot root exudate. Exudate from Ri T-DNA transformed carrot roots was concentrated and semipurified on a SEPAK C18 cartridge and injected into Petri plates containing pregerminated *Gigaspora gigantea* spores. Arbuscular-like branching was observed after 15 hr. This type of branching required the presence of 2% CO₂. Insert is a higher magnification of the arbuscule-like branches.

may be important in this type of experiment.

Cellular signals (exudates or root mucilage) may be necessary before appressoria of AM fungi are formed [1, 18], and the lack of signals in nonhost exudate prevents appressoria formation [16]. Topographical or biochemical signals on the root surface also are thought to be necessary for appressoria to form [1] and recently some compounds hydrolyzed from cell walls have been shown to stimulate hyphal growth [19].

Nagahashi and Douds [20] used *in vitro* culture techniques to address the question of whether the formation of appressoria is governed by

cellular signals or topographical signals.

Transformed carrot roots can be infected and colonized by the AM fungi, *G. gigantea* and *G. margarita*. Cell walls from cultured carrot roots (host) and sugar beets (nonhost) were isolated and purified with the Parr N₂ bomb procedure [21] that allows for the recovery of very large cell wall fragments which retain biological activity [20, 22]. Their results clearly showed that both *G. gigantea* and *G. margarita* form appressoria on isolated host epidermal cell wall pieces in the absence of exudate signals, protoplasts, or intact cells [20]. The epidermal cell walls could be distinguished from

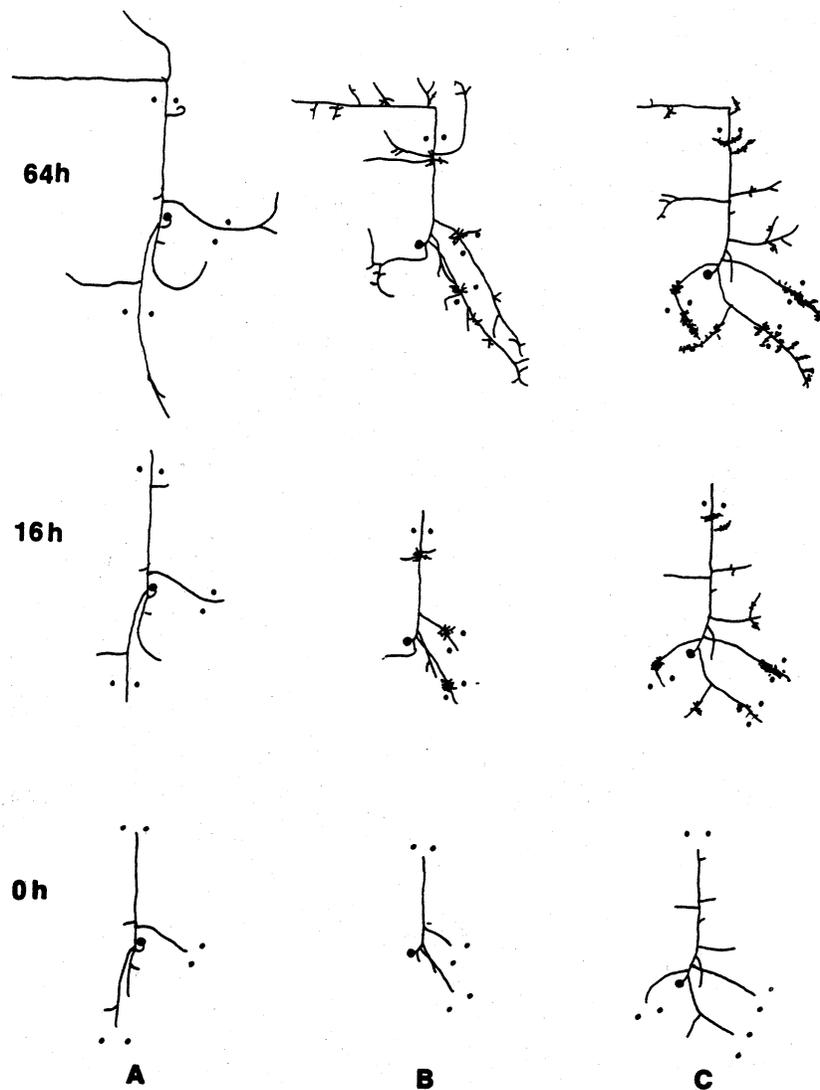


Figure 3. Time course of hyphal branching of germinated spores of *Gigaspora gigantea* after exposure to exudate isolated from Ri T-DNA transformed carrot roots. The branching signals were concentrated and semipurified on a SEPAK C18 cartridge and injected near growing hyphal tips. The growth was traced after various periods of time. A. Control injected with sterilized water. B. Injected with concentrated exudate diluted 1:100. C. Injected with concentrated exudate.

cortical and vascular cell walls (Figure 4).
Appressoria did not form on any isolated wall
fragments of the nonhost sugarbeet roots.
The successful formation of appressoria on

purified cell walls can be attributed to the ability to
concentrate the cell walls in a Petri dish which
increased the chances of hyphal interaction with a
host cell wall fragment [20]. In addition, the Parr

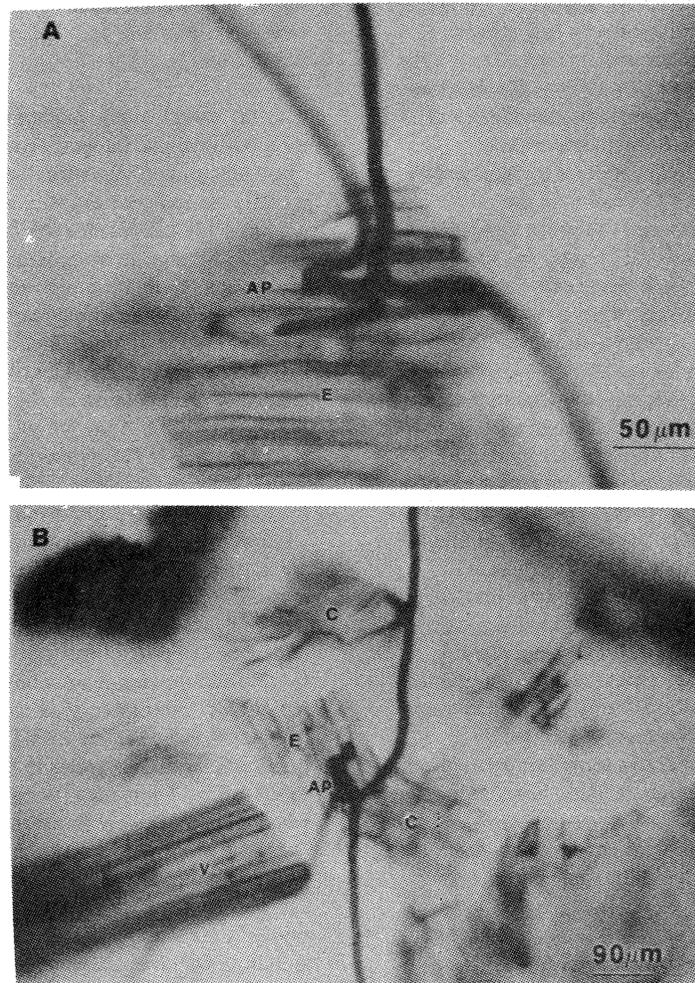


Figure 4. Micrographs of appressoria formation on isolated and purified epidermal cell wall fragments. Hyphae from *Gigaspora gigantea* were allowed to grow and interact with cell walls isolated from Ri T-DNA transformed carrot roots. A. Appressorium formation occurs in the groove between adjacent epidermal cells. B. Appressorium formation on epidermal cell walls (E) which can be distinguished from cortical cell walls (C) and vascular cell walls (V).

N₂ bomb isolation procedure allowed for the recovery of isolated cell wall fragments up to 5-6 epidermal cells long which retained their normal surface topology between cells. The commonly used cell wall isolation procedures would have probably been unsuccessful because these fragments are typically less than one cell length [23, 24] and the surface topology between cells is destroyed.

Since appressoria formed on isolated cell walls in the absence of exudate, it was clear that exudate signals or cytoplasmic signals for attachment and subsequent appressoria formation were not necessary. The most frequent place for appressoria formation on intact root surfaces is in the longitudinal groove between two adjacent epidermal cells [17]. This was also the primary site for appressoria formation on isolated cell walls [20]. This suggests that topology of the groove is probably essential for hyphae to differentiate and hence the requirement for specific topographical signals as well as biochemical components present at the specific binding sites. Although specific topographical signals of a host were reported for growth orientation and appressorium formation of a parasitic fungus, *Uromyces appendiculatus* [25], this type of study has not been done for AM fungus-host associations. The use of purified host cell walls in plate cultures has opened this avenue of research so that physical as well as biochemical properties of the binding site can eventually be determined. Fungal attachment and recognition led to appressorium formation during parasitic invasion of a host plant [26]. Whether AM fungal attachment and appressorium formation are two separate events for this nonparasitic interaction is unclear at this time. It has not been clearly demonstrated that an attached hyphae may not form an appressorium during AM fungal host root interactions.

The experiments with isolated cell walls also produced another interesting observation. Appressoria formation was not followed by penetration. Penetration buds formed on epidermal wall pieces but the penetration hyphae became septate. This suggested that an intact epidermal cell or a cytoplasmic signal from an epidermal or cortical cell was necessary for penetration to occur. The need for a penetration signal also has been

demonstrated by the isolation of "early" myc-mutants which allow appressoria formation but do not allow penetration and further hyphal growth [27].

Information on host-fungus signaling gained from the study of legume mutants and host defense reactions: evidence for signaling after root penetration

"It is evident that the highly sophisticated, morphofunctional integration between AM symbionts can only be explained by synchronized modification in gene expression of both the partners" [28]. Evidence for the exchange of signals whereby the host regulates the development of the fungus and the fungus regulates aspects of the physiology of the host root comes from mutants of legumes unable to form functioning nodules and mycorrhizae. Further evidence is provided by studies of plant defense responses regulated by AM fungi.

Duc et al [27] first reported on mutants of *Pisum sativum* and *Vicia faba* which were unable to form nodules (nod-) and mycorrhizae (myc-). These mutants also exist for *Medicago sativa* [29]. There are at least 6 genetic loci in pea which can be mutated to form nod- [27], while mutations at only four of those loci can produce myc- [30]. This allows for the possibility of the nod- myc+ genotype [31]. This common regulation lead to the belief that flavonoids, important signal molecules in the legume-*Rhizobium* symbiosis [32], were also important signal molecules in the AM symbiosis [33, 34, 35]. These ideas were later discounted when flavonoids were found to be unnecessary for the establishment of arbuscular mycorrhizae [36].

Myc- mutants of legumes fall into two categories: those which arrest development of mycorrhizae after appressoria formation ("early") and those which allow penetration and intercellular hyphal growth but subsequently inhibit arbuscule formation ("late") [37]. Despite the formation of intraradical hyphae by "late" mutants, there is no evidence of carbon transport to the fungus [38]. These mutants indicate at least two steps in the recognition/signaling/regulation process during penetration and colonization of roots. First, a

recognition step requiring genetic control is necessary for the plant to allow fungal penetration following appressoria formation. Early myc-mutants exhibit cell wall thickening right under appressoria and these callose deposits inhibit penetration [39]. Secondly, once penetration has occurred and intercellular hyphal growth begins, further signaling/recognition under genetic control is necessary for the penetration of cortical cells and formation of arbuscules. This is indicated by the late myc- mutants and could explain the lack of penetration hyphal structures on isolated cortical cell walls [20].

Another aspect of communication between host and AM fungus is in the regulation of host defense mechanisms in the mycorrhiza. The invading AM fungus must inhibit or evade the host defense system. It has been reported that genes encoding for chitinases, β -1,3-glucanases, and enzymes for the synthesis of other defense related compounds are activated upon infection of plant tissue by invading fungi [40]. Mycorrhizal fungi cause a different response from the host's defense system [41, 42]. Alfalfa roots inoculated with the AM fungus *G. intraradices* exhibited a transient increase in activities of chalcone isomerase, chitinase, and upon colonization, phenylalanine ammonia lyase [43]. The activity of these enzymes were then suppressed to levels below those of uninoculated controls. Activity of isoflavone reductase, important for the synthesis of the phytoalexin medicarpin, was shown to decrease after colonization of alfalfa roots by *Glomus versiforme* to levels below those of controls, but this decrease did not occur in myc- plants [44]. Recent evidence suggests the decline in levels of defense-related enzymes is due to suppression of defense-related gene expression [45, 46, 47]. A recent review [48] indicated that a mild induction of defense-response gene expression continues to occur as the AM fungus grows through the root. It was further suggested that the AM fungus does not elicit a general signal through the root system to completely suppress the host defense system but the recognition process must be initiated with each new cell contact to result in suppression of the defense response. Recent evidence also indicates a root may distinguish between AM fungi [49]. Transformed

roots of *M. sativa* exhibited a hypersensitive-like response when penetrated by *G. margarita* but allowed colonization by *G. intraradices*. These results along with the activation and suppression of host defense genes indicates a two-way signaling event between fungus and host.

Extraradical hyphal development and sporulation after colonization

It is not clear how much extraradical hyphal growth occurs after the initial appressorium formation and penetration but before arbuscule formation. It is also not known to what degree the hyphae exiting the plant root versus hyphae attached to the original spore (or external hyphae attached to a penetration hyphae) make up the extraradical mycelium. It is known that the extraradical hyphae entering the root are different in diameter and wall thickness compared to hyphae leaving the root surface [50]. It is also known that the extraradical hyphae are structurally and functionally different [51] compared to the intraradical hyphae. This was demonstrated by use of the split plate technology [52] where an infected host root was allowed to grow in a Petri dish that was separated into two halves. The infected root grows in one compartment and eventually an extraradical hypha grows over the barrier (plastic septum) into the second compartment. The physical barrier prevents the growing root from crossing over and lateral or secondary root growth can be trimmed and prevented from crossing the septum. The barrier also prevents exchange by diffusion between the solid media present in each half. Compounds can be applied either to the colonized root side or to the extraradical hyphal side and uptake studies can be performed. Conditions in the nonroot side of the plate also can be modified and exogenous compounds can be added to study the effects of signals on the development of the extraradical hyphae. It is also possible that signals are not involved and the development of the extraradical hyphae may be a natural consequence of the newly acquired nutritional status of the fungus.

Spores usually develop on the extraradical hyphae and the production of new spores represents the completion of the life cycle of AM fungi.

However, the role of signaling in sporulation by AM fungi is largely unexplored. Because localized areas of abundant sporulation have been observed in dual cultures *in vitro*, it has been suggested that if such a signal exists [53] it would be carried cytoplasmically through the fungus inducing differential morphogenesis in certain hyphae. Alternatively, extraradical hyphae can have several origins and may therefore respond differently to internal or external signals.

Sporulation may not be stimulated by signals *per se* since spore populations in natural environments typically increase as host plants senesce [54, 55, 56]. In this case, the accumulation of secondary metabolites could act as a negative signal by inhibiting hyphal growth. The fungus also may perceive the declining availability of photosynthate as a signal to produce overwintering spores. The onset of sporulation can also coincide with the attainment of a critical colonized root length in experimental situations where the host plant does not senesce [57, 58]. This also indicates sporulation may be regulated by the availability of photosynthate in another way, *i.e.* sporulation may not begin until the availability of carbon surpasses a critical level. Variation in the availability of photosynthate with different host plants, and different requirements/responses by different AM fungi, may explain the host species dependent sporulation noted for many AM fungi [59, 60]. Whether the communication events between fungus and host during extraradical hyphal growth and sporulation are one-way or two-way events has not yet been determined.

CONCLUSIONS

Sequential stages of signaling/recognition/communication must occur for an AM fungus to complete its life cycle in the presence of a host root. Germination can occur under appropriate environmental conditions but all further steps leading to colonization of a host root involve some form of signaling (Figure 5). The first signal perceived by the fungus is constitutive to the host and is exuded by the root. This host signal is not induced by the fungus because it is produced by

roots which have never been exposed to the fungus. This signal causes hyphal growth and elongation and when concentrated near a root surface, a rapid and profuse branching of hyphal tips can occur. The presence of multiple signals and possible synergistic effects between various signals is consistent with the lack of specificity between AM fungi and host roots. Although a signal (and there may be several different chemical categories and types of signals) has not yet been identified, the major function appears to be the growth and proliferation of branches which would increase the chance of contact with a root surface.

The second step is appressoria formation which is a contact recognition event between a growing fungal hypha and the epidermal surface of a host root. This also appears to be a one-way communication event since appressoria can form on isolated and purified host epidermal cell walls in the absence of an intact cell. The branching signal(s) in the exudate would increase the chances of hyphal contact with a specific host cell wall binding site. The possibility that the increased branching also has an assimilatory role [1] should not be neglected.

The lack of penetration hyphae after appressoria formation on isolated walls indicates a third signaling or recognition step is necessary and this is consistent with the existence of "early" myc-mutants. It is not known whether penetration and intercellular spread of the hyphae are stimulated by the same signal. We have combined these steps for the purpose of the model presented in Figure 5. After hyphae reach the cortical parenchyma, differentiation of intracellular arbuscules is induced (step 4).

The third and fourth steps would appear to be two-way communication events. Penetration and intercellular spread of fungal hyphae require stimulation from the host but at the same time, the fungus must inhibit or evade the host defense system. During arbuscule formation, the fungal wall must contact and penetrate a cortical cell wall. Whether specific binding of penetrating hyphae to a cortical cell wall site is necessary has not yet been determined. The arbuscular cell walls are highly modified and it has been suggested that the host root affects fungal metabolism in such a fashion that arbuscules are fungal walls that have failed to

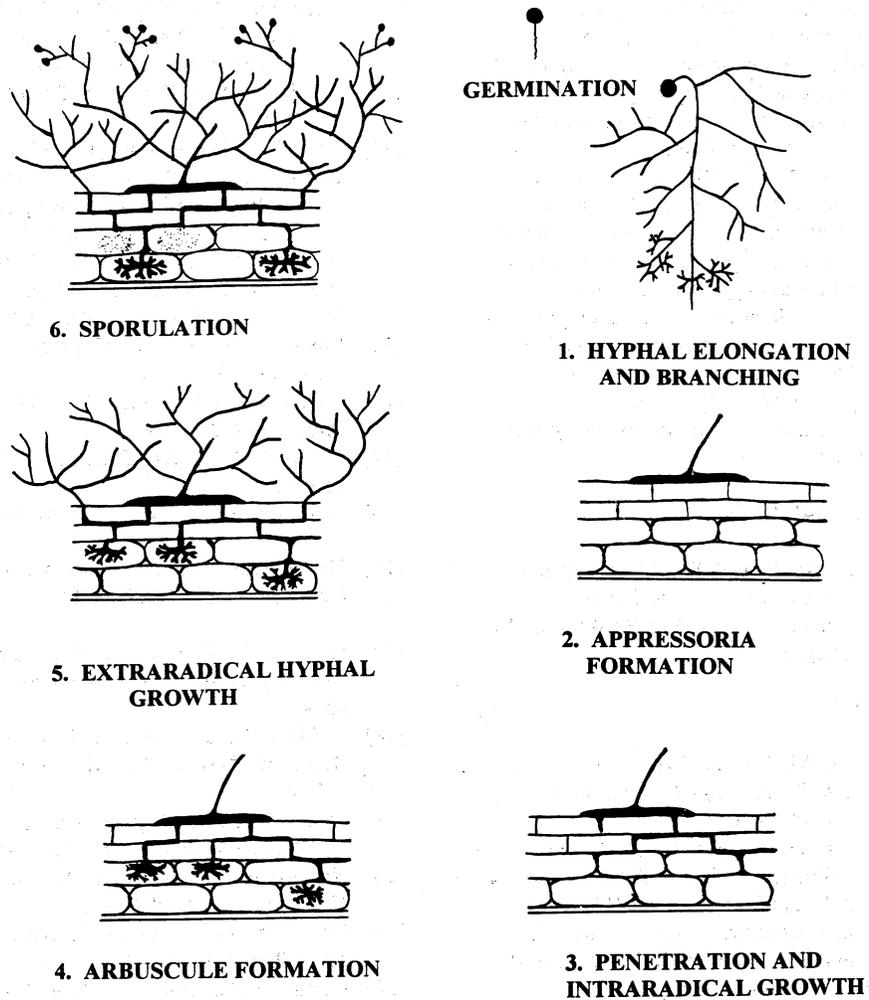


Figure 5. A model of the signaling/recognition steps between a germinated AM fungal spore and colonization of a host root. The first two steps are simply a fungal response to the host and both can now be completed *in vitro* without the presence of a living root. The third and fourth steps are two-way communication steps between the fungus and host. The fifth and sixth steps are extraradical hyphal growth and sporulation and whether or not these steps are two-way communication events has not yet been determined.

mature [41].

After arbuscule formation, there is an extensive proliferation of external mycelium which may arise due to the change in nutritional status of

the fungus but could also be triggered by a host signal. Proliferation of these hyphae could be considered a distinct fifth step since recent evidence indicates that the extraradical hyphae are not the

same functionally as the intraradical hyphae even though they are directly connected [51].

We consider sporulation as the sixth and final step in the life cycle and this step may occur simply as a result of the acquired nutritional status of the fungus, the stage of colonization by the fungus, or the age of the host root. For *Gigaspora* species, sporulation occurs on the extraradical hyphae and not the intraradical hyphae. Either some compound inhibits the intraradical hyphae from sporulating or some compound could trigger the extraradical hyphae to sporulate. In any case, the development of the split plate technology [52] has provided a system to test whether or not the addition of chemical compounds or changes in environmental conditions can stimulate extraradical hyphal growth and/or sporulation.

ACKNOWLEDGEMENT

We would like to thank Dr. Roger T. Koide (Pennsylvania State University) and Professor Manuela Giovannetti (Università Di Pisa) for kindly reviewing this chapter.

REFERENCES

- [1] Giovannetti, M., Sbrana, Avio, L., C., Citeresi, A. S., and Logi, C. 1993, *New Phytol.* 125, 587-593.
- [2] Douds, D. D. Jr., and Schenck, N. C. 1991, *Soil Biol. Biochem.*, 23, 177-183.
- [3] Bonfante, P., and Bianciotto, V. 1995, *Mycorrhiza: Structure, function, molecular biology and biotechnology*, B. Varma, and B. Hock (Eds.), Springer-Verlag, Berlin Heidelberg, 229-247. 747p.
- [4] Koske, R. E. 1982, *Transactions of the British Mycological Society*, 79, 305-310.
- [5] Koske, R. E., and Gemma, J. N. 1992, *Mycorrhizal Functioning: An Integrative Plant-Fungal Process*, M. F. Allen (Ed.), Chapman and Hall, publishers, New York, NY., 3-36.
- [6] Bécard, G., and Piché, Y. 1989, *Appl Environ Microbiol.*, 55, 2320-2325.
- [7] Graham, J. H. 1982, *Mycologia*, 68, 831-835.
- [8] Elias, K. E., and Safir, G. R. 1987, *Appl. Environm. Microbiol.*, 53, 1928-1933.
- [9] Nagahashi, G., Douds, D. D., and Abney, G. D. 1996, *Mycorrhiza*, 6, 403-408.
- [10] Douds, D. D. Jr. 1994, *New Phytol.*, 126, 233-237.
- [11] Bécard, G., Beguiristain, T., and Nagahashi, G. 1997, *Current Topics in Plant Physiology*, vol 18: *Radical Biology: Advances and Perspectives on the Function of Plant Roots*, H. Flores, J. P. Lynch, and D. Eissenstat, (Eds.), American Society of Plant Physiologists, Rockville, MD, 164-177.
- [12] Mosse, B., and Hepper, C. 1975, *Physiol. Plant Pathol.*, 5, 215-223.
- [13] Bécard, G., and Fortin, J. A. 1988, *New Phytol.* 108, 211-218.
- [14] Mosse, B. 1988, *Can. J. Botany*, 66, 2533-2540.
- [15] Staples, R. C., Macko, V., 1980, *Exp. Mycol.*, 4, 2-16.
- [16] Giovannetti, M., Avio, L., Sbrana, C. and Citeresi, A. S. 1993, *New Phytol.* 123, 115-122.
- [17] Garriock, M. L., Peterson, R. L., and Ackerley, C. A. 1989, *New Phytol.*, 112, 85-92.
- [18] Koide, R. T., and Schreiner R. P. 1992, *Ann Rev Plant Physiol. Plant Mol. Biol.*, 43, 557-81.
- [19] Douds, D. D. Jr., Nagahashi, G., and Abney, G. 1996, *New Phytol.*, 133, 289-294.
- [20] Nagahashi, G., and Douds, D. D. Jr. 1997, *New Phytol.*, 136, 299-304.
- [21] Nagahashi, G., Abney, G. D., and Uknalis, J. 1994, *Protoplasma*, 178, 129-137.
- [22] Nagahashi, G., Seibles, T. 1986, *Protoplasma*, 134, 102-110.
- [23] Codignola, A., Verotta, L., Spanu, P., Maffei, M., Scannerini, S., and Bonfante-Fasolo, P. 1989, *New Phytol.*, 112, 221-228.
- [24] Nagahashi, G., Seibles, T. S., Jones, S. B., and Rao, J. 1985, *Protoplasma*, 129, 36-43.
- [25] Hoch, H. C., Staples, R. C., Whitehead, D., Comeau, J., and Wolf, E. D. 1987, *Science*, 235, 1659-62.
- [26] Manocha, M. S., and Chen, Y. 1990, *Can. J. Microbiol.*, 36, 69-76.
- [27] Duc, G., Trouvelot, A., Gianinazzi-Pearson,

- V., and Gianinazzi, S. 1989, *Plant Sci.*, 60, 215-212.
- [28] Gianinazzi-Pearson, V., Gollotte, A., Lherminier, J., Tisserant, B., Franken, P., Dumas-Gaudot, E., Lemoine, M.-C., van Tuinen, D., and Gianinazzi, S. 1995, *Can. J. Bot.*, (Suppl 1) S526-S532.
- [29] Bradbury, S. M., Peterson, R. L., and Bowley, S. R. 1991, *New Phytol.*, 119, 115-120.
- [30] Bonfante, P., and Perotto, S. 1995, *New Phytol.*, 130, 3-21.
- [31] Bradbury, S. M., Peterson, R. L., and Bowley, S. R. 1993, *New Phytol.*, 124, 665-673.
- [32] Peters, N. K., and Verma, D. P. S. 1990, *Mol. Plant Microbe Interact.*, 3, 4-8.
- [33] Bécard, G., Douds, D. D., and Pfeffer, P. E. 1992, *Appl. Environ. Microbiol.*, 58, 821-825.
- [34] Chabot, S., Bel-Rhliid, R., Chenevert, R., and Piché, Y. 1992, *New Phytol.*, 122, 461-467.
- [35] Phillips, D. A., and Tsai, S. M. 1992, *Mycorrhiza*, 1, 55-58.
- [36] Bécard, G., Taylor, L. P., Douds, D. D., Pfeffer, P. E., and Doner, L. W. 1995, *Mol. Plant Microbe Interact.*, 8, 252-258.
- [37] Gianinazzi-Pearson, V., Gianinazzi, S., Guillemin, J. P., Trouvelot, A., and Duc, G. 1991, *Advances in Molecular Genetics of Plant-Microbe Interactions. Vol I*. H. Hennecke, D. P. S. Verma, (Eds.), Kluwer Academic Press, Dordrecht, Boston, London, 336-346.
- [38] Kling, M., Gianinazzi-Pearson, V., Lherminier, J., and Jakobsen, I. 1996, *Proceedings of the First International Conference on Mycorrhizae. August 4-9, 1996; Berkeley, CA, USA*, 71.
- [39] Gollotte, A., Gianinazzi-Pearson, V., Giovannetti, M., Sbrana, C., Avio, L., and Gianinazzi, S. 1993, 191, 112-122.
- [40] Bowles, D. J. 1990, *Ann. Rev. Biochem.*, 59, 873-907.
- [41] Gianinazzi-Pearson, V., Dumas-Gaudot, E., Gollotte, A., Tahiri-Alaoui, A., and Gianinazzi, S. 1996, *New Phytol.* 133, 45-57.
- [42] Kapulnik, Y., Volpin, H., Itzhaki, H., Ganon, D., Galili, S., David, R., Shaul, O., Elad, Y., Chet, I., and Okon, Y. 1996, *New Phytol.*, 133, 59-64.
- [43] Volpin, H., Elkind, Y., Okon, Y., and Kapulnik, Y. 1994, *Plant Physiol.*, 104, 683-689.
- [44] Harrison, M. J., and Dixon, R. A. 1993, *Mol Plant Microbe Interact.* 6, 643-654.
- [45] Volpin, H., Phillips, D. A., Okon, Y., and Lapulnik, Y. 1995, *Plant Physiol.*, 108, 1449-1454.
- [46] Lambais, M. R., and Mehdy, M. C. 1996, *New Phytol.*, 134, 531-538.
- [47] David, R., Itzhaki, H., Ginzberg, I., Gafni, Y., Galili, G., and Kapulnik, Y. 1998, *Molecular Plant Microbe Interactions*, 11, 489-497.
- [48] Barker, S. J., Tagu, D., and Delp, G. 1998, *Plant Physiol.*, 116, 1201-1207.47.
- [49] Douds, D.D. Jr., Galvez, L., Becard, G., and Kapulnik, Y. 1998, *New Phytol.*, 138;27-35.
- [50] Friese, C. F., and Allen, M. F. 1991, *Mycologia*, 83, 409-418.
- [51] Pfeffer, P. E., Shachar-Hill, Y., Becard, G., Rolin, D., and Douds, D. D. Jr. 1998, *Current Topics in Plant Physiology*, Vol. 18, H. E. Flores, and J. P. Lynch, (Eds.); *Radical Biology: Advances and Perspectives on the Function of Plant Roots*. American Society of Plant Physiologists, Rockville, MD., 187-209.
- [52] St-Arnaud, M., Hamel, C., Vimard, B., Caron, M., and Fortin, J. A. 1996, *Mycol. Res.*, 100, 328-332.
- [53] Bago, B., Azcon-Aguilar, C., and Piche, Y. 1998, *Mycologia*, 90, 52-62.
- [54] An, Z. Q., and Hendrix, J. W. 1993, *Soil Biol. Biochem.*, 25, 813-817.
- [55] Dhillon, S. S., and Anderson, R. C. 1993, *Can. J. Bot.*, 71, 1625-1630.
- [56] Lee, P. J., and Koske, R. E. 1994, *Mycol. Res.*, 98, 453-457.
- [57] Gazey, C., Abbott, L. K., and Robson, A. D. 1992, *Mycol. Res.*, 96, 643-650.
- [58] Heldreth, K., and Morton, J. B. 1996, *Proceedings of the First International Conference on Mycorrhizae, August 4-9, 1996; Berkeley, CA, USA*, 61.
- [59] Hetrick, B. A. D., and Bloom, J. 1986, *Mycologia*, 78, 32-36.
- [60] Bever, J. D., Morton, J. B., Antonovics, J., and Schultz, P. A. 1996, *J. Ecol.*, 84, 71-82.