APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1999, p. 5571–5575 Vol. 65, No. 12
0099-2240/99/$04.00+0

Anastomosis Formation and Nuclear and Protoplasmic Exchange in Arbuscular Mycorrhizal Fungi

MANUELA GIOVANNETTI,* DARIO AZZOLINI, AND ANNA SILVIA CITERNESI

Dipartimento di Chimica e Biotecnologie Agrarie, Centro di Studio per la Microbiologia del Suolo, CNR, Università di Pisa, 56124 Pisa, Italy

Received 13 May 1999/Accepted 17 September 1999

We observed anastomosis between hyphae originating from the same spore and from different spores of the same isolate of the arbuscular mycorrhizal fungi *Glomus mosseae*, *Glomus caledonium*, and *Glomus intraradices*. The percentage of contacts leading to anastomosis ranged from 35 to 69% in hyphae from the same germling and from 34 to 90% in hyphae from different germlings. The number of anastomoses ranged from 0.6 to 1.3 per cm (length) of hyphae in mycelia originating from the same spore. No anastomoses were observed between hyphae from the same or different germlings of *Gigaspora rosea* and *Scutellospora castanea*; no interspecific or intergeneric hyphal fusions were observed. We monitored anastomosis formation with time-lapse and video-enhanced light microscopy. We observed complete fusion of hyphal walls and the migration of a mass of particles in both directions within the hyphal bridges. In hyphal bridges of *G. caledonium*, light-opaque particles moved at the speed of 1.8 ± 0.06 μm/s. We observed nuclear migration between hyphae of the same germling and between hyphae belonging to different germlings of the same isolate of three *Glomus* species. Our work suggests that genetic exchange may occur through intermingling of nuclei during anastomosis formation and opens the way to studies of vegetative compatibility in natural populations of arbuscular mycorrhizal fungi.

Arbuscular mycorrhizal (AM) fungi are obligate symbionts that live in association with the roots of most land plants and play a major role in nutrient uptake and in interplant nutrient transfer (8, 27, 39). In experimental microcosms and in the field, AM hyphal connections between roots are important for the maintenance of stability and biodiversity in plant communities (32, 36). Little is known about the dynamics of hyphal growth during presymbiotic and symbiotic phases (24, 30, 31), and virtually nothing is known of the ability of these fungi to form the hyphal networks through which nutrients are proposed to flow. Although anastomoses occur widely between vegetative hyphae of ascomycetes and basidiomycetes (1, 3, 17, 21), they are believed to be lacking or rare in zygomycetes (5, 16), to which AM fungi belong. The occurrence of anastomosis in AM fungi has been mentioned by some authors (11, 15, 26, 40), but no quantitative data are available on the frequency of hyphal fusions in the different species, and, to our knowledge, no information has been published on the cytological events involved.

In this study we monitored anastomosis between hyphae derived from individually germinated spores of AM fungi via a combination of time-lapse and video-enhanced light microscopy, image analysis, and epifluorescence microscopy. Our main objectives were (i) to monitor anastomosis formation in living hyphae; (ii) to detect cytoplasmic flow and nuclear exchange between anastomosing hyphae; (iii) to determine the occurrence and frequency of anastomosis between hyphae belonging to the same and to different germinated spores of the same isolate of *Glomus mosseae*, *Glomus caledonium*, *Glomus intraradices*, *Gigaspora rosea*, or *Scutellospora castanea*; and (iv) to determine the kind of interaction occurring between hyphae belonging to different genera (*Glomus* versus *Gigaspora* and *Gigaspora* versus *Scutellospora*) and to different species of the same genus (*Glomus*).

* Corresponding author. Mailing address: Dipartimento di Chimica e Biotecnologie Agrarie, Via del Borghetto 80, 56124 Pisa, Italy. Phone: 39-050-571561. Fax: 39-050-571562. E-mail: mgiova@agr.unipi.it.

MATERIALS AND METHODS

Fungal material. Spores of AM fungi were obtained from pot cultures maintained in the collection of the Department of Chemistry and Agricultural Biotechnology, University of Pisa, Pisa, Italy. The AM fungi used were *G. mosseae* (Nicolson et Gerdemann) Gerdemann et Trappe (Kent isolate) (Banque Européenne des Glomales [BEG] code 12), *G. caledonium* (Nicolson et Gerdemann) Trappe et Gerdemann (Rothamsted isolate) (BEG 20), *G. intraradices* Schenck et Smith, isolated from Bourgogne, France (LPA 8), *G. intraradices* Schenck et Smith, isolated from Liguria, Italy (IMA 5), *Glomus viscosum* Nicolson (BEG 27), *G. rosea* Nicolson et Gerdemann (BEG 9), and *S. castanea* Walker (BEG 1). Inocula of the *G. intraradices* French isolate, *G. rosea*, and *S. castanea* were kindly provided by V. Gianinazzi-Pearson, Laboratoire de Phytoparasitologie, Dijon, France.

Dynamics of anastomosis formation in living hyphae. Spores of *G. caledonium* and *G. mosseae* were surface sterilized with 2% chloramine T supplemented with streptomycin (400 μg ml^{-1}) for 20 min and then rinsed five times in sterile distilled water. The spores were germinated on 20- by 20-mm cellophane membranes (Hoefer, San Francisco, Calif.) and placed on a thin layer of 1% water agar in 5.5-cm-diameter petri dishes for direct observations of living hyphae under a Reichert-Jung (Vienna, Austria) Polyvar microscope equipped with differential interference contrast optics and epifluorescence optics. We monitored anastomosis formation in hyphal tips showing directed growth towards nearby hyphae, which were growing at a distance of about 70 μm. To visualize the establishment of protoplasmic continuity and the viability of anastomosed hyphae, succinate dehydrogenase (SDH) activity was assessed on germinated spores, which were stained, mounted on microscope slides, and observed for the presence of formazan salt depositions in hyphal bridges (38).

Nuclear migration and cytoplasmic flow through anastomoses. We mounted cellophane membranes bearing germinated spores of *G. caledonium* and *G. mosseae* on microscope slides in sterile distilled water. The coverslips were sealed with 1% water agar, which was periodically wetted with sterile distilled water. The microchambers obtained were transferred to the Polyvar microscope and observed for 2 h. Bright contrast images were acquired after regulation of the condenser diaphragm. The experiments were carried out at room temperature (19 to 21°C). To monitor nuclei in hyphae during anastomosis formation, 7- to 14-day-old germinated spores were mounted on microscope slides in diaminophenylindole (DAPI) (Sigma, St. Louis, Mo.) at 5 μg/ml of microscopy 1:1 water-glycerol and were observed under epifluorescence by using the filter combination U1 (BP 330-380, LP 418, DS 420). Images were acquired with a Reichert 100× oil immersion lens with a 1.25 numerical aperture. Time-lapse and video-enhanced light microscopy was used to obtain images, which were captured with a 3 charge-coupled device color video camera connected to a

TABLE 1. Anastomosis rate in mycelia originating from the same spore or from different spores of the same isolate in five species of AM fungi and numbers of anastomoses and hyphal contacts per centimeter (length) of hypha in mycelia originating from the same spore

Fungal species	% Anastomosis[a] (mean and 95% exact confidence limits) between:		No. of anastomoses/cm (length) of hypha (mean ± SE)	No. of hyphal contacts/cm (length) of hypha (mean ± SE)
	HSS	HDS		
G. mosseae BEG 12				
Culture 14.93	57 (48–66)		0.65 ± 0.11	1.2 ± 0.19
Culture 44.93	51 (42–61)	40 (31–49)	0.95 ± 0.23	2.2 ± 0.76
G. caledonium BEG 20				
Culture 156.92	55 (46–63)	34 (27–41)**	0.62 ± 0.06	1.2 ± 0.14
Culture 5.91	35 (30–39)		1.1 ± 0.1	3.4 ± 0.37
Culture 163.92	48 (43–53)	34 (24–44)*	1.1 ± 0.3	2.3 ± 0.59
G. intraradices				
French isolate LPA 8	59 (51–66)		1.3 ± 0.23	2.3 ± 0.34
Italian isolate IMA 5	69 (62–75)	90 (81–99)**	1.1 ± 0.18	1.7 ± 0.29
G. rosea			0	1.7 ± 0.14
S. castanea			0	3.2 ± 0.23

[a] Anastomosis as a percentage of contacts. HSS, hyphae from the same spore. HDS, hyphae from different spores. Within the same fungal species, values of HDS marked with one or two asterisks are significantly different from the corresponding values of HSS at $P < 0.05$ or $P < 0.01$, respectively.

videocassette recorder (Hi 8, EV-C2000E; SONY, Tokyo, Japan). Selected frames were printed on a video graphic printer (UP-890 CE; SONY).

Occurrence and frequency of anastomoses. Spores were rinsed five times in sterile distilled water and were allowed to germinate individually in sterile distilled water in microliter plates (Sigma). Germinated spores were transferred to Millipore membranes (0.45-μm-diameter pores), which were placed on moist, sterile quartz sand in 9-cm-diameter petri dishes. After 7 to 14 days of incubation in the dark at 28°C, mycelium growing on the membranes was stained with Trypan Blue (0.05% in lactic acid), mounted on microscope slides, and observed under the Polyvar light microscope. Hyphal length was assessed by using Quantimet 500 image analysis software (Leica, Milan, Italy). Pairings of individually germinated spores were made by placing germinated spores approximately 1 cm apart on the membrane filter. Findings are based on at least 10 germinated spores for anastomoses within the same germling, 9 pairings for anastomoses between different germlings from the same isolate, and 9 sets of pairings for interspecific and intergeneric anastomoses. Replicate experiments were carried out on spores produced in different pot cultures (Table 1). The frequency of anastomosis was calculated by determining the proportion of hyphal contacts which had anastomosed. Chi-square analysis was used to determine the homogeneity of anastomosis frequency data, and the chi-square test of independence was performed to detect significant differences in anastomosis frequency between hyphae from the same spores and hyphae from different spores.

RESULTS

Dynamics of anastomosis formation in living hyphae. Spatiotemporal studies made it possible to monitor anastomosis formation, which took about 35 min after a hyphal tip showed directed growth towards another hypha, in *G. caledonium* and *G. mosseae* mycelia. Complete fusion of hyphal walls and cytoplasmic flow between fused hyphae were observed. We observed protoplasmic continuity, the characteristic feature of true vegetative hyphal fusion, as SDH activity in all the anastomoses. Depositions of formazan salt were detected in anastomosing hyphae and in hyphal bridges (Fig. 1).

Nuclear migration and cytoplasmic flow through anastomoses. DAPI staining and epifluorescence microscopy showed that hyphal anastomosis involved the migration of nuclei via the fusion bridge. Migration occurred between hyphae belonging to the same germling (Fig. 2a and b) and between hyphae belonging to different germlings of the same isolate (Fig. 2c and d).

We used time-lapse and video-enhanced microscopy to detect protoplasmic streaming inside the hyphae and to monitor the trajectory of a mass of particles (e.g., vacuoles, mitochondria, nuclei, and fat droplets) migrating in both directions within the protoplasm and through anastomoses (Fig. 3). The mass streaming of particles occurred at a speed of 1.8 ± 0.06 μm/s (mean ± standard error of the mean [SEM]) in hyphal bridges of *G. caledonium*.

Occurrence and frequency of anastomoses. We observed anastomoses between hyphae belonging to the same germinated spore and between different spores of the same isolate in all three *Glomus* species. The percentage of anastomoses between hyphae from the same germling ranged from 35% in *G. caledonium* to 69% in the Italian isolate of *G. intraradices* (Table 1), and the number of anastomoses ranged from 0.62 ± 0.06 (mean ± SEM) to 1.3 ± 0.23 per cm (length) of hyphae (Table 1). Hyphal fusions also occurred readily between hyphae belonging to different germlings from the same isolate. The anastomosis rate ranged from 34% in *G. caledonium* to 90% in the Italian isolate of *G. intraradices* (Table 1).

No anastomoses were observed between hyphae belonging to the same germling of *G. rosea* or *S. castanea* (Table 1). No fusions were detected in more than 220 hyphal contacts between germlings of *G. rosea*. No fusions were detected in more than 460 hyphal contacts between germlings of *S. castanea*.

No anastomoses were observed in pairings between germlings of different species of AM fungi: *G. mosseae* and *G. caledonium* (0 fusions, 90 contacts), *G. mosseae* and *G. rosea* (0 fusions, 140 contacts), *G. caledonium* and *G. rosea* (0 fusions,

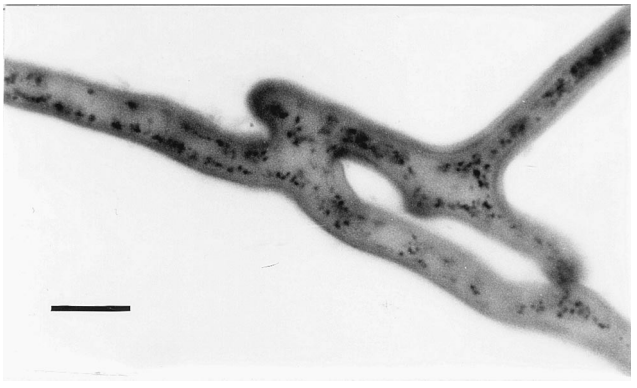

FIG. 1. Micrograph showing complete fusion of hyphal walls and the establishment of protoplasmic continuity in two anastomosing hyphae of *G. mosseae*, visualized by histochemical localization of SDH activity. Depositions of formazan salt are evident in hyphal bridges. Bar, 10 μm.

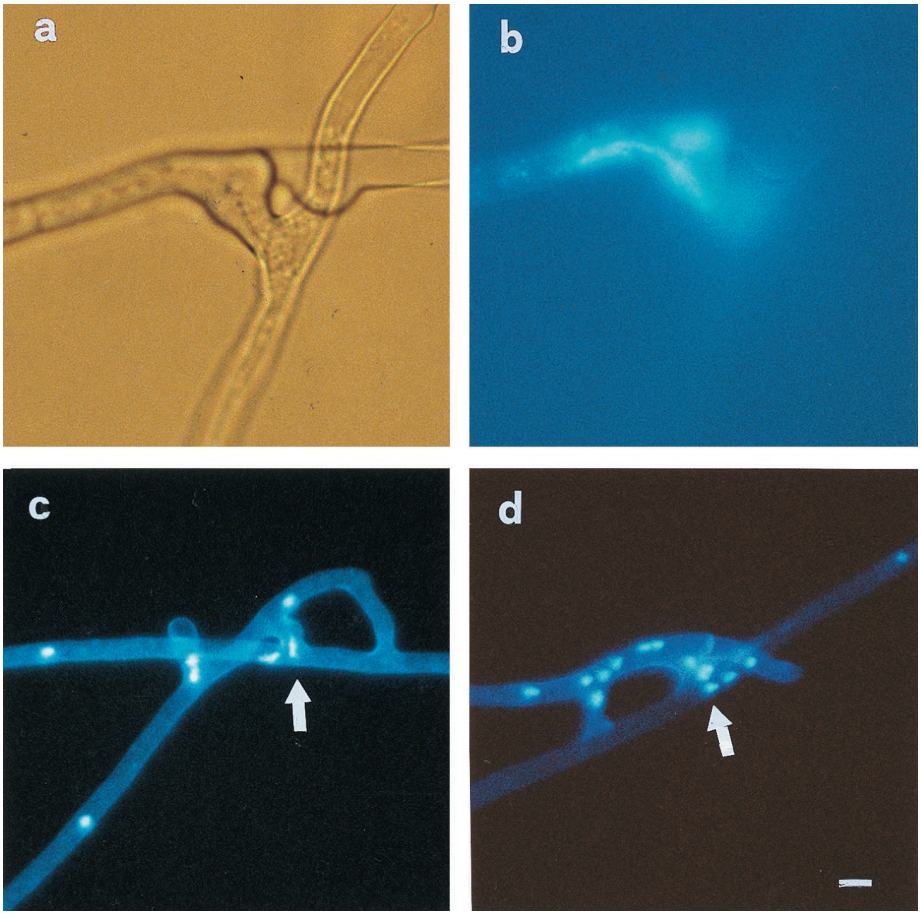

FIG. 2. Localization of nuclear migration between two anastomosing hyphae of *G. caledonium* belonging to the same germling (a and b) and to different germlings of the same isolate (c and d). (a) Light micrograph illustrating the site of hyphal fusion. (b) Epifluorescence image of the same field, showing an elongated nucleus (stained with DAPI) in the middle of the fusion bridge. Bar, 7 μm. (c and d) Epifluorescence microscopy of the nuclei within fusion bridges (arrows). Bar, 12 μm.

232 contacts), and *G. rosea* and *S. castanea* (0 fusions, 98 contacts).

During interspecific and intergeneric hyphal interactions, the responses ranged from no contact interference, when hyphae simply appeared to overgrow each other, to contact responses such as formation of hyphal swellings devoid of protoplasm and septate (Fig. 4) or growth of one hypha along the other without anastomosis formation.

DISCUSSION

Anastomosis formation and the cytological events involved have been studied extensively in ascomycetes and basidiomycetes (1, 3, 17, 21, 25). Anastomoses are believed to be lacking or rare in zygomycetes (5, 16), although their occurrence in AM fungi has been mentioned by others (11, 15, 26, 40). In this report, we present evidence for anastomosis formation between hyphae originating from the same spore and from different spores in one isolate of *G. mosseae*, one isolate of *G. caledonium*, and two different isolates of *G. intraradices*. The frequency of anastomoses per contacts in hyphae from the same germling was comparable to that found within self-anastomosing isolates of *Rhizoctonia solani*—more than 50% (19). The number of anastomoses per centimeter (length) of hypha ranged from 0.62 to 1.3, corresponding to 0.22 to 0.45 fusions per mm^2, as worked out from hyphal density data (13). These values are lower than those observed in fungi that are able to grow saprophytically, such as *Gibberella fujikuroi*—6.9 to 8.1 fusions per mm^2 in self-compatible strains (6)—but still significant when the poor growth ability of the presymbiotic mycelium of AM fungi is considered (2, 24).

In the three *Glomus* species, hyphal fusions occur regularly in mycelia originating from different spores. In *G. mosseae* the frequency of anastomosis formation between hyphae from different germlings was not statistically different from that for mycelia originating from the same germling, whereas *G. intraradices* and *G. caledonium* showed significantly higher and lower frequencies of anastomosis per contact, respectively, in hyphae originating from different spores. No anastomoses were observed between hyphae from the same or different germlings of *G. rosea* and *S. castanea*, and this characteristic may be another difference between the Glomineae and Gigasporineae families (37).

During interspecific and intergeneric interactions, no hyphal fusions were detected, suggesting that hyphae recognize species-level differences and confirming previous qualitative findings (40). The ability to discriminate self from other at the intraspecific level, however, remains to be demonstrated. The use of tests based on vegetative compatibility may lead to the identification of genetically different isolates in population studies of pathogenic, saprophytic, and ectomycorrhizal fungi (3, 7, 9, 20–22, 29, 35). Our work opens the way to studies of vegetative compatibility in natural populations of AM fungi.

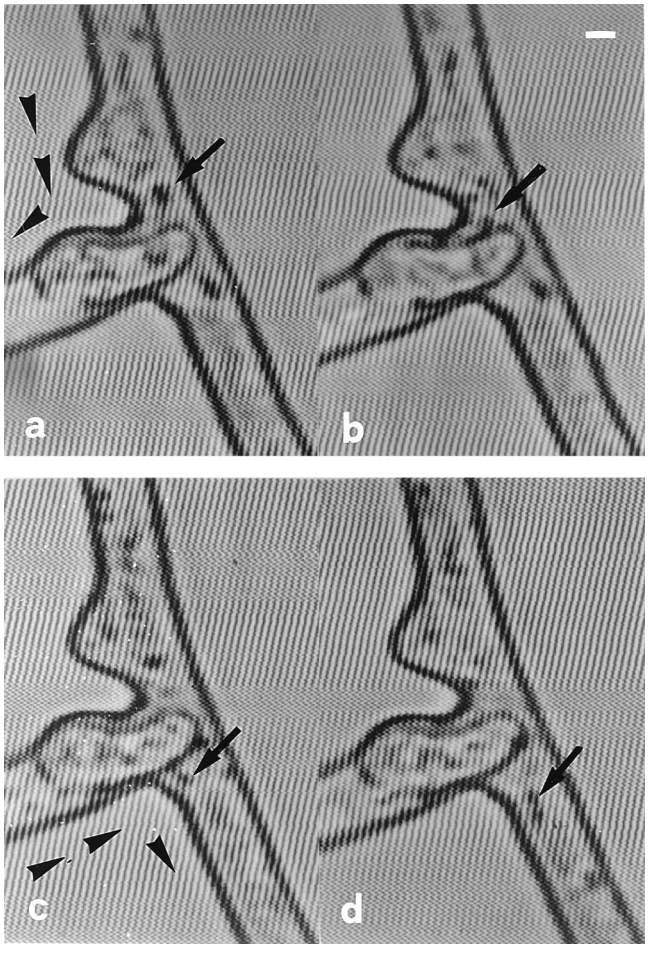

FIG. 3. Protoplasmic flow subsequent to anastomosis in *G. caledonium*, visualized over time by video-enhanced light microscopy. Cytoplasmic continuity is established between two fused hyphae, evidenced by the bidirectional movement of particles (arrowheads). (a and b) A large, light-opaque particle migrating from one hypha to the other via the fusion bridge (arrows). Time sequence: 0 (a) and 2 (b) s. (c and d) Two coupled light-opaque particles migrating in the opposite direction, via the fusion bridge (arrows). Time sequence: 0 (c) and 2 (d) s. Bar, 3.8 μm.

For example, different isolates of *G. mosseae*, a species of worldwide distribution, could be paired with known tester strains to determine if they are genetically isolated as indicated by vegetative compatibility.

AM fungi are ancient obligate biotrophs (18) which, although lacking host-regulated spore germination (14), have survived for 400 million years (28, 33, 37). We suggest that the ability of germlings to form anastomoses with compatible hyphae may affect the fitness of *Glomus* species; the young mycelium produced by spores germinating in the absence of the host may connect to a mycelial network as soon as the germ tube contacts a compatible mycelium, increasing its chance to colonize host roots. The formation of anastomoses also can restore protoplasmic continuity in damaged hyphae (10).

We obtained direct evidence for nuclear exchange between hyphae from the same germling and between hyphae of different germlings of the same isolate in three *Glomus* species. Nuclei from single spores of *Glomus* species may be heterogeneous, based on variation in molecular characters (23, 34, 41). Since *Glomus* spores are multinucleate and may contain 1,000 to 5,000 nuclei per spore (4, 12, 42), some researchers have suggested that these spores are heterokaryotic (23, 34). Our

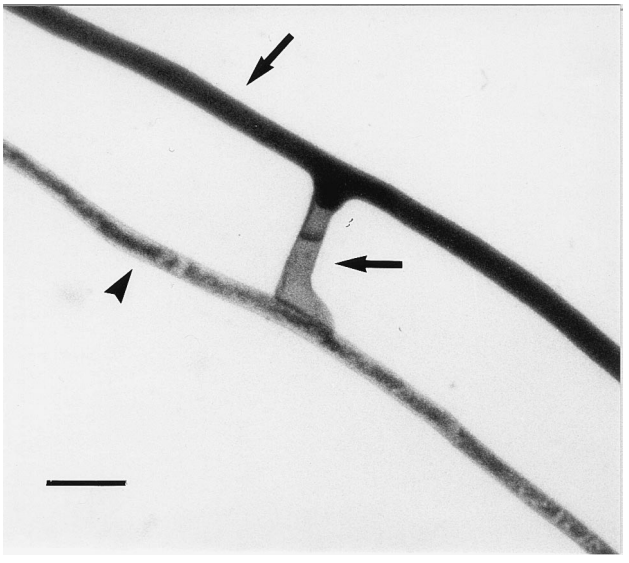

FIG. 4. Interactions between hyphae originating from two different species of AM fungi, *G. mosseae* (upper arrow) and *G. viscosum* (arrowhead). Note the formation of a hyphal swelling which becomes empty and septate without any anastomosis formation (lower arrow). Bar, 20 μm.

results suggest that genetic exchange may occur through intermingling of nuclei during anastomosis formation between different germlings of the same isolate. This exchange could result in information flow, as well as physiological and genetic integration, between mycelia belonging to different germlings.

Lack of knowledge of the genetics of AM fungi and our inability to culture them in axenic culture make it difficult to discern the mechanism by which genetic exchange occurs. Further studies are needed in order to understand how these obligate symbionts maintain genetic diversity within spores.

ACKNOWLEDGMENT

This work was partly supported by a University of Pisa grant (Fondo di Ateneo).

REFERENCES

1. **Ainsworth, A. M., and A. D. M. Rayner.** 1986. Responses of living hyphae associated with self and non-self fusions in the basidiomycete *Phanerochaete velutina*. J. Gen. Microbiol. **132:**191–201.
2. **Becard, G., and Y. Piché.** 1989. Fungal growth stimulation by CO_2 and root exudates in vesicular-arbuscular mycorrhizal symbiosis. Appl. Environ. Microbiol. **55:**2320–2325.
3. **Brasier, C.** 1992. A champion thallus. Nature **356:**382–383.
4. **Burggraaf, A. J. P., and J. E. Beringer.** 1988. Nuclear division and VA-mycorrhizal in vitro culture, p. 190. *In* D. M. Sylvia, L. L. Hung, and J. H. Graham (ed.), Mycorrhizae in the next decade. University of Florida, Gainesville.
5. **Carlile, M. J.** 1995. The success of the hypha and mycelium, p. 3–19. *In* N. A. R. Gow and G. M. Gadd (ed.), The growing fungus. Chapman & Hall, London, United Kingdom.
6. **Correll, J. C., C. J. R. Klittich, and J. F. Leslie.** 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). Mycol. Res. **93:**21–27.
7. **Dahlberg, A., and J. Stenlid.** 1994. Size, distribution and biomass of genets in populations of *Suillus bovinus* (L.: Fr.) Roussel revealed by somatic incompatibility. New Phytol. **128:**225–234.
8. **Francis, R., and D. J. Read.** 1984. Direct transfer of carbon between plants connected by vesicular-arbuscular mycorrhizal mycelium. Nature **307:**53–56.
9. **Fries, N.** 1987. Somatic incompatibility and field distribution of the ectomycorrhizal fungus *Suillus luteus* (Boletaceae). New Phytol. **107:**735–739.
10. **Gerdemann, J. W.** 1955. Wound-healing of hyphae in a phycomycetous mycorrhizal fungus. Mycologia **47:**916–918.
11. **Giovannetti, M., L. Avio, C. Sbrana, and A. S. Citernesi.** 1993. Factors affecting appressorium development in the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe. New Phytol. **123:** 115–122.

12. **Giovannetti, M., and V. Gianinazzi-Pearson.** 1994. Biodiversity in arbuscular mycorrhizal fungi. Mycol. Res. **98:**705–715.
13. **Giovannetti, M., C. Sbrana, L. Avio, A. S. Citernesi, and C. Logi.** 1993. Differential hyphal morphogenesis in arbuscular mycorrhizal fungi during preinfection stages. New Phytol. **125:**587–593.
14. **Giovannetti, M., C. Sbrana, and C. Logi.** 1994. Early processes involved in host recognition by arbuscular mycorrhizal fungi. New Phytol. **127:**703–709.
15. **Godfrey, R. M.** 1957. Studies on British species of *Endogone*. III. Germination of spores. Trans. Br. Mycol. Soc. **40:**203–210.
16. **Gregory, P. H.** 1984. The fungal mycelium—an historical perspective, p. 1–22. *In* D. H. Jennings and A. D. M. Rayner (ed.), The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, United Kingdom.
17. **Gregory, P. H.** 1984. The fungal mycelium: an historical perspective. Trans. Br. Mycol. Soc. **82:**1–11.
18. **Hepper, C. M.** 1983. Limited independent growth of a vesicular-arbuscular mycorrhizal fungus *in vitro*. New Phytol. **93:**537–542.
19. **Hyakumachi, M., and T. Ui.** 1987. Non-self-anastomosing isolates of *Rhizoctonia solani* obtained from fields of sugarbeet monoculture. Trans. Br. Mycol. Soc. **89:**55–159.
20. **Legrand, P., S. Ghahari, and J. J. Guillaumin.** 1996. Occurrence of genets of *Armillaria* spp. in four mountain forests in Central France: the colonization strategy of *Armillaria ostoyae*. New Phytol. **133:**321–332.
21. **Leslie, J. F.** 1993. Fungal vegetative compatibility. Annu. Rev. Phytopathol. **31:**27–150.
22. **Leslie, J. F.** 1991. Mating populations in *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology **81:**1058–1060.
23. **Lloyd-MacGilp, S. A., S. M. Chambers, J. C. Dodd, A. H. Fitter, C. Walker, and J. P. W. Young.** 1996. Diversity of the ribosomal internal transcribed spacers within and among isolates of *Glomus mosseae* and related mycorrhizal fungi. New Phytol. **133:**103–111.
24. **Logi, C., C. Sbrana, and M. Giovannetti.** 1998. Cellular events involved in survival of individual arbuscular mycorrhizal symbionts growing in the absence of the host. Appl. Environ. Microbiol. **64:**3473–3479.
25. **McCabe, P. A., M. P. Gallagher, and J. W. Deacon.** 1999. Microscopic observation of perfect hyphal fusion in *Rhizoctonia solani*. Mycol. Res. **103:** 487–490.
26. **Mosse, B.** 1959. The regular germination of resting spores and some observations on the growth requirements of an *Endogone* sp. causing vesicular-arbuscular mycorrhiza. Trans. Br. Mycol. Soc. **42:**273–286.
27. **Newman, E. I., and W. R. Eason.** 1993. Rates of phosphorus transfer within and between ryegrass (Lolium perenne) plants. Funct. Ecol. **7:**242–248.
28. **Phipps, C. J., and T. N. Taylor.** 1996. Mixed arbuscular mycorrhizae from the Triassic of Antarctica. Mycologia **88:**707–714.
29. **Rayner, A. D. M.** 1991. The challenge of the individualistic mycelium. Mycologia **83:**48–71.
30. **Read, D. J.** 1984. The structure and function of the vegetative mycelium of mycorrhizal roots, p. 215–240. *In* D. H. Jennings and A. D. M. Rayner (ed.), The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, United Kingdom.
31. **Read, D. J.** 1984. The mycorrhizal mycelium, p. 102–133. *In* M. F. Allen (ed.), Mycorrhizal functioning. An integrative plant-fungal process. Chapman & Hall, New York, N.Y.
32. **Read, D. J.** 1997. The ties that bind. Nature **388:**517–518.
33. **Remy, W., T. N. Taylor, H. Hass, and H. Kerp.** 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc. Natl. Acad. Sci. USA **91:** 11841–11843.
34. **Sanders, I. R., M. Alt, K. Groppe, T. Boller, and A. Wiemker.** 1995. Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. New Phytol. **130:**419–427.
35. **Sen, R.** 1990. Intraspecific variation in two species of *Suillus* from Scots pine (*Pinus sylvestris* L.) forests based on somatic incompatibility and isozyme analyses. New Phytol. **114:**603–612.
36. **Simard, S. W., D. A. Perry, M. D. Jones, D. D. Myrold, D. M. Durall, and R. Molina.** 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. Nature **388:**579–582.
37. **Simon, L., J. Bousquet, R. C. Levesque, and M. Lalonde.** 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature **363:**67–69.
38. **Smith, S. E., and V. Gianinazzi-Pearson.** 1990. Phosphate uptake and arbuscular activity in mycorrhizal Allium cepa L.: effects of photon irradiance and phosphate nutrition. Aust. J. Plant Physiol. **17:**177–188.
39. **Smith, S. E., and D. J. Read.** 1996. Mycorrhizal symbiosis. Academic Press, San Diego, Calif.
40. **Tommerup, I. C.** 1988. The vesicular-arbuscular mycorrhizas. Adv. Plant Pathol. **6:**81–91.
41. **Vandenkoornhuyse, P., and C. Leyval.** 1998. SSU rDNA sequencing and PCR-fingerprinting reveal genetic variation within *Glomus mosseae*. Mycologia **90:**791–797.
42. **Viera, A., and M. G. Glenn.** 1990. DNA content of vesicular-arbuscular mycorrhizal fungal spores. Mycologia **82:**263–267.