thiamine (100 μg/l) and by varying light conditions. The best results were obtained on MEA, irrespective of thiamine addition, by incubation at 25° in darkness for 7 days followed by further growth at 20–21° under natural diffused light for 6 days. Under these conditions a zone of moderately heavy sporulation 10–15 mm wide was produced near the margin.

The distinguishing features of *Umbelopsis fusiformis* are its fusiform sporangia and its requirement for light to sporulate. *U. fusiformis* is closely related to *Mortierella rammanniana*. Sporangiospore colour and shape are similar in both species but columella development differs. The former species has indistinct columellae whereas those of the latter species are well-developed, although minute in size. The distinct sporangial shape of *U. fusiformis*, however, sets it apart from *M. ramanniana* and all other species in subgenus *Micromucor*.

Umbelopsis fusiformis appears to be a rare fungus in the soils of the forest at Wallaby Creek. The two sites which yielded the fungus are *ca* 500 m apart. Soils from undisturbed sites under a mature and a young forest were also studied, but the fungus was not found in these soils.

This work was completed at the Botany School, University of Melbourne and I wish to thank Drs G. Weste, H. J. Swart and D. H. Ashton for a critical reading of the manuscript. Invaluable advice on taxonomy given by Dr Walter Gams of C.B.S., Baarn, The Netherlands, is greatly appreciated. I am grateful to Mr J. C. Martyn of the Department of Classical Studies, University of Melbourne for rendering the Latin diagnosis. Research grants provided by the Melbourne Metropolitan Board of Works and the Forests Commission of Victoria are gratefully acknowledged.

REFERENCES

AINSWORTH, G. C. (1971). Ainsworth and Bisby's *Dictionary of the Fungi* 6th ed. Kew: Commonwealth Mycological Institute.

ARX, J. A. v. (1982). On Mucoraceae s.str. and other families of the Mucorales. *Sydowia* **35**, 10–36.

BLAKESLEE, A. F. (1915). Linder's roll tube method of separation culture. *Phytopathology* **5**, 68–69.

GAMS, W. (1977). A key to the species of *Mortierella*. *Persoonia* **9**, 381–391.

KORNERUP, A. & WANSCHER, J. H. (1975). *Methuen Handbook of Colour*, 3rd ed. London: Eyre Methuen.

PARKINSON, D. & WILLIAMS, S. T. (1961). A method for isolating fungi from soil microhabitats. *Plant and Soil* **4**, 347–355.

TURNER, M. (1963). Studies in the genus *Mortierella*. I. *Mortierella isabellina* and related species. *Transactions of the British Mycological Society* **46**, 262–272.

EFFECT OF SOIL MICRO-ORGANISMS ON SPORE GERMINATION AND GROWTH OF THE VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS *GLOMUS MOSSEAE*

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Germination rate of surface-sterilized chlamydospores of the vesicular-arbuscular (VA) mycorrhizal endophyte *Glomus mosseae* was significantly hastened by the presence of free-living fungi in a time-course experiment on water-agar. The fungi also stimulated hyphal growth, while vegetative spores formed only on the mycelium arising from resting spores grown in the presence of such free-living micro-organisms. The time of appearance of vegetative spores was related to the time when the free-living fungi were added. The interest in soil biology and ecology of these microbe–microbe relationships involving VA fungi is discussed.

Substances known to be present in the plant rhizosphere can enhance the axenic growth of VA fungal hyphae (Hepper, 1979; Graham, 1982; Siqueira, Hubbel & Schenk, 1982; Hepper, 1983; Hepper & Jakobsen, 1983). The role of other factors from the rhizosphere, e.g. soil micro-organisms, needs further study.

The influence of such organisms on VA spore germination was pointed out by Mosse (1959) and Mejstrik (1965). More recently Daniels & Trappe (1980) noted that the loss of micro-organisms inhibited spore germination, and Barea & Azcón-Aguilar (1982) indicated that the presence of some micro-organisms was able to stimulate the non-symbiotic development of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe (Gerdemann & Trappe, 1974). These observations, together with recent descriptions supporting a direct stimulatory effect

of soil microbiota on the saprophytic, pre-infective stages of *G. mosseae* in soil (Azcón-Anguilar & Barea, 1985), justify further studies on the topic.

The present paper reports assays aimed to test the effect of a number of micro-organisms on development of *G. mosseae*, to select the more effective ones and to study their influence on germination and growth by means of a time-course experiment.

The experiments were conducted in 9 cm diam plastic Petri dishes containing water-agar (1 % Difco Bacto agar) adjusted to pH 7·0. Sporocarps of *G. mosseae* were obtained from rhizospheres of *Medicago sativa* L. plants grown in stock-pot cultures in the glasshouse. The rhizosphere samples were kept for some months in polyethylene bags at 4 °C and after collection sporocarps were stored on damp filter paper at 4 °. Resting spores freshly isolated from these sporocarps by excision were surface sterilized in Chloramine T, streptomycin and Tween 80 mixture (Mosse, 1962) for 20 min and then washed five times in sterile water. Spores were transferred by sterile capillary pipettes to Petri dishes. Incubation was at 25 ° in the dark, the plates being sealed with parafilm to reduce dehydration and contamination risks.

In the first experiment, 20–25 surface-sterilized spores per plate were placed near (1–2 cm) the edge of the Petri dish and, at least 6 cm away from spores, a thin streak of each one of the free-living micro-organisms to be assayed was inoculated in five replicate plates. These micro-organisms, as yet unidentified, were isolated from soil by standard procedures. They were grown in agar slope cultures, and small pieces of the microbial colonies were dispersed in sterile water and used as inocula.

Germination rate and hyphal growth of *G. mosseae* were assessed after 4 weeks incubation. A spore was considered germinated if a germ-tube was clearly visible. To evaluate the effect of the screened free-living micro-organisms on growth, four categories, namely (i), (ii), (iii) and (iv) were established, according to the length of the hyphae (measured as the largest dimension of the area covered) and the presence of vegetative spores on the mycelium arising from germinating resting spores. These categories are defined and illustrated in Fig. 1.

The fungi F_3 and F_4 were selected for a further experiment. Six surface-sterilized spores of *G. mosseae* were transferred individually to each Petri dish, located on the vertices of an imaginary hexagon of about 3·5 cm side. The free-living fungi, F_3 and F_4, were inoculated as a small portion of their mycelia at the centre of the dish, equidistant from the six *G. mosseae* spores, after 0, 4, 8 and 12 days. Each treatment had five replicate plates, and five were kept as controls with the *G. mosseae* spores growing axenically. Spore germination and hyphal development were periodically examined under a light microscope.

Despite the fact that no nutrient was added to the culture medium, the free-living micro-organisms, mainly the fungi, exhibited some development. The bacterial colonies spread slightly and the fungi grew between 2·2 (F_4) and 12·5 (F_3) mm per day.

All the free-living micro-organisms, even without contact, enhanced the development of the VA fungus (Fig. 1). Spore germination, which was only assessed after 4 weeks in this experiment, was apparently unaffected by microbial inoculation, reaching between 90 and 95 % in all cases including the axenically grown spores. Nevertheless, the further time-course experiment demonstrated an improvement in the rate of germination which was significant for some treatments (Table 1). Isolates F_3 and F_4 not only achieved a successful enhancement of the rate of *G. mosseae* spore germination (Table 1) but also improved the subsequent mycelial growth, particularly the elongation of the hyphae, and the production of small vegetative spores. The number of spores was significantly different from that in the control in most observations (Table 2). The time of appearance of the small vegetative spores was dependent on the inoculation time of the free-living fungi and also, but to a lesser extent, on the fungal isolate, since F_3 triggered the process earlier than F_4 but F_4 seems to promote the formation of a higher number of vegetative spores than F_3.

There is overwhelming evidence that greater than 90 % germination of *Glomus* spores can be obtained axenically on 1 % water-agar. The present results confirm that *G. mosseae* spores readily germinated axenically. Nevertheless, the germination rate was hastened by the presence of free-living micro-organisms through a still unknown mechanism. These micro-organisms also stimulated subsequent hyphal growth. Numbers of vegetative spores formed per germinated resting spore were similar to those found for *G. caledonium* by externally applied organic substances (Hepper, 1979). These, mainly vitamins and amino acids, are known to be excreted to the medium by soil micro-organisms (Lynch, 1976). Since the free-living fungi tested in this paper were able to stimulate *G. mosseae* when growing some distance from it, the possible role of a volatile or highly diffusible substance cannot be excluded. Conversely, the reported microbial activity might be based on the utilization by the free-living organisms of some self-inhibitors of *Glomus* spore germination, the presence of which was suggested by Watrud, Heithaus & Jaworski (1978) and the microbial role

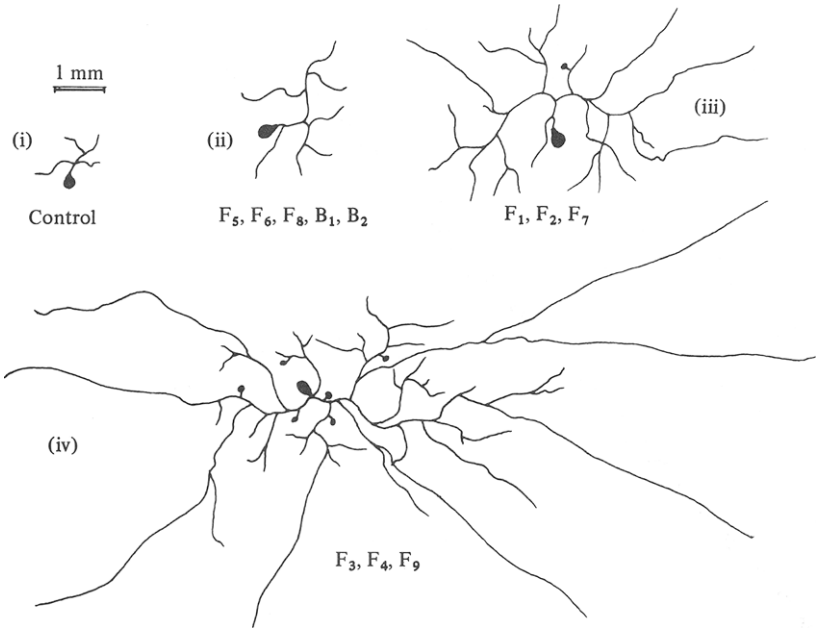

Fig. 1. Typical development of germinating resting spores of *G. mosseae* on water-agar, as affected by the presence of free-living fungi (F_n) and bacteria (B_n). Four categories were recognized according to the extent of hyphal growth and the production of vegetative spores, after a 4-week period of incubation: (i) negligible: the largest dimension was less than 2 mm and no vegetative spores formed; (ii) slight: between 2 and 5 mm, no vegetative spores present; (iii) moderate: between 5 and 10 mm and few vegetative spores; (iv) extensive: greater than 10 mm, with abundant vegetative spores formed. The size of axenically grown controls, (i), and the most predominant features of development under the influence of the micro-organisms F_{1-9} and B_{1-2} are shown.

Table 1. *Percentage germination of* G. mosseae *spores in the presence of two free living fungi (F_3 and F_4) on water agar*

Fungus	Inoculation time (days)	Time (days) 6	8	11	13	16	20	26	32
Uninoculated control	—	3·4	7·3	17·4	35·3	64·0	83·0	87·2	91·9
F_4	0	4·7	4·7	34·0*	**53·2***	81·0*	90·2	95·2	97·6
	4	2·2	6·7	20·0	46·0	78·0	**85·7**	91·8	91·8
	8	—	—	35·9**	62·5**	84·1**	92·1	**95·3**	98·4
	12	—	—	—	38·9	78·4	92·0	**94·0**	95·9
F_3	4	0·0	**2·2**	19·6	63·0**	95·6***	97·8**	97·8*	100·0*
	8	—	—	**20·0**	53·7*	87·8**	95·1*	95·1	95·1
	12	—	—	—	32·6	**75·6**	92·5	100·0**	100·0*

Bold figures indicate when the contact between the free-living fungi and *G. mosseae* hyphae took place in each treatment.

*, **, *** Significantly different from the uninoculated control at the 5%, 1% and 0·1% levels respectively.

Table 2. *Effect of two free-living fung (F_3 and F_4) on formation of vegetative spores on mycelium arising from resting spores of* G. mosseae

Fungus	Inoculation time (days)	Number of vegetative spores per germinated resting spore*						
		8	11	13	16	20	26	32
Uninoculated control	—	0	0	0	0	0	0	0
F_4	0	0	0·9±1·2	**2·4±1·3**	3·7±1·0	4·2±1·0	4·7±1·0	4·9±1·0
	4	0	0	0	2·6±1·0	**4·3±0·7**	4·6±0·7	4·6±0·7
	8	—	0	0·3±0·4	1·2±0·5	3·5±0·6	**4·8±0·3**	5·4±0·6
	12	—	—	0	0	0·2±0·1	**2·8±0·5**	4·4±0·6
F_3	4	0	0	0·7±0·5	2·3±0·6	2·8±0·5	3·1±0·4	3·2±0·4
	8	—	0	0·4±0·3	2·0±0·6	3·1±0·5	3·3±0·5	3·4±0·5
	12	—	—	0	**0·3±0·2**	3·3±0·6	3·6±0·5	3·9±0·6

Bold figures indicate when the contact between the free-living fungi and *G. mosseae* took place in each treatment.
* Mean value ± confidence limit at 5% level of significance.

in their removal indicated by Daniels & Trappe (1980).

The substances that have been reported to increase the hyphal growth from *Glomus* spores are usually present in the plant rhizosphere and may be produced by soil micro-organisms, which in turn are known to be stimulated in the root region. The possibility that the reported effects could also take place under natural conditions is reinforced by the observation by Azcón-Aguilar & Barea (1985) indicating that common soil micro-organisms can account for an improvement of the growth of *G. mosseae* in soil.

These results therefore give direct evidence of microbe–microbe mutualistic relationships, involving VA fungi, that could be of interest in soil biology and ecology.

This work has been supported by CAICyT Project no. 1759/82 and is dedicated to Prof. L. Leloir on his 80th birthday.

REFERENCES

AZCON-AGUILAR, C. & BAREA, J. M. (1985). Effect of soil micro-organisms on formation of vesicular-arbuscular mycorrhizas. *Transactions of the British Mycological Society* **84**, 536–537.

BAREA, J. M. & AZCON-AGUILAR, C. (1982). Interactions between mycorrhizal fungi and soil microorganisms. In *Les Mycorhizes: Biologie et Utilisation* (ed. S. Gianinazzi, V. Gianinazzi-Pearson & A. Trouvelot), pp. 181–193. Paris: INRA publ.

DANIELS, B. A. & TRAPPE, J. M. (1980). Factors affecting spore germination of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeus*. *Mycologia* **72**, 457–471.

GERDEMANN, J. W. & TRAPPE, J. M. (1974). The Endogonaceae in the Pacific Northwest. *Mycologia Memoir* **5**, 1–76.

GRAHAM, J. H. (1982). Effect of citrus root exudates on germination of chlamydospores of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeum*. *Mycologia* **74**, 831–835.

HEPPER, C. M. (1979). Germination and growth of *Glomus caledonius* spores: the effects of inhibitors and nutrients. *Soil Biology and Biochemistry* **11**, 269–277.

HEPPER, C. M. (1983). Limited independent growth of a vesicular-arbuscular mycorrhizal fungus *in vitro*. *New Phytologist* **93**, 537–542.

HEPPER, C. M. & JAKOBSEN, I. (1983). Hyphal growth from spores of the mycorrhizal fungus *Glomus caledonius*: effect of amino acids. *Soil Biology and Biochemistry* **15**, 55–58.

LYNCH, J. M. (1976). Products of soil micro-organisms in relation to plant growth. *CRC Critical Reviews in Microbiology* **5**, 67–107.

MEJSTRIK, J. (1965). Study of the development of endotrophic mycorrhiza in the association of *Cladietum marisci*. In *Plant Microbe Relationships* (ed. J. Macura & V. Vancura), pp. 283–290. Prague: Czechoslovak Academy of Science.

MOSSE, B. (1959). The regular germination of resting spores and some observations on the growth requirements of an *Endogone* sp. causing vesicular-arbuscular mycorrhiza. *Transactions of the British Mycological Society* **42**, 273–286.

MOSSE, B. (1962). The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. *Journal of General Microbiology* **27**, 509–520.

SIQUEIRA, J. O., HUBBEL, D. H. & SCHENCK, N. C. (1982). Spore germination and germ tube growth of a vesicular-arbuscular mycorrhizal fungus *in vitro*. *Mycologia* **74**, 952–959.

WATRUD, L. S., HEITHAUS III, J. J. & JAWORSKI, E. G. (1978). Evidence for production of inhibitor by the vesicular-arbuscular-mycorrhizal fungus *Gigaspora margarita*. *Mycologia* **70**, 821–828.