Axenic Germination of Vesicular-Arbuscular Mycorrhizal Fungi: Effects of Selected *Streptomyces* Species

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ABSTRACT

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The influence of *Streptomyces avermitilis, S. griseus*, and *S. orientalis* on germination of surface-disinfested *Gigaspora margarita, Glomus mosseae*, and *Scutellispora heterogama* spores on 1.5% Noble water agar (WA) was studied. *G. margarita* and *G. mosseae* germination was stimulated by *S. orientalis*. Spores germinated more frequently on double-layer WA with *S. orientalis* microcolonies suspended in the bottom layer than on WA. Experiments conducted with four-compartment petri plates indicated that stimulation was due to one or more volatile compounds. Germination of *G. mosseae* spores was also stimulated by *S. avermitilis* and *S. griseus* on double-layer WA but was generally poor on WA not containing *Streptomyces* microcolonies. *G. mosseae* spore germination on WA was not influenced by pH. *S. heterogama* spore germination was suppressed by *S. avermitilis* and *S. orientalis* on double-layer WA but was stimulated by *S. orientalis* in four-compartment petri plates when the two organisms were in separate quadrants. Generally, germination of *S. heterogama* spores was inversely related to WA pH. All three *Streptomyces* spp. increased pH of WA overlayers by two pH units after 9 days at 25 C.

Additional keyword: soil microorganisms.

The obligately symbiotic nature of vesicular-arbuscular mycorrhizal (VAM) fungi has precluded precise physiological and genetic study of these organisms in the laboratory. Consequently, culture of VAM fungi on excised bindweed (26), carrot (8), clover (24), and tomato (22) roots on holidic media has been utilized as an alternative to axenic culture. Pregerminated, surface-disinfested VAM fungal spores are routinely used to inoculate excised root cultures. However, germination of such spores under axenic conditions is frequently unpredictable, slow, or nonexistent (10,11,15,21,25,32).

Many biotic and abiotic factors affect the germination of azygospores and chlamydospores of VAM fungi. Inorganic ion, nutrient, and vitamin concentrations, as well as aeration, light, moisture, pH, and temperature influence the frequency of VAM fungal spore germination in vitro and in soil (29). Response of spores to these factors varies among fungal genera, species, and, with some factors, isolates.

Inhibition and stimulation of VAM fungal spore germination have also been associated with the activities and metabolic products of other soil microorganisms. Germination of *Glomus etunicatum* Becker & Gerdemann and *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe chlamydospores was suppressed in nonsterile soil relative to spore germination in autoclaved and pasteurized soils (32). Furthermore, addition of nonsterile soil sievings retarded the germination of these VAM fungal spores in the heat-treated soils. *Acaulospora laevis* Gerdemann & Trappe, *Gigaspora calospora* (Nicol. & Gerd.) Gerdemann & Trappe, and *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerdemann spore germination was also suppressed in two natural soils (30). The inhibition was alleviated by heating or methyl bromide fumigation. Water-soluble, heat-labile compounds extracted from one of the natural soils also suppressed *G. caledonium* spore germination on water agar.

Conversely, VAM fungal spore germination can be stimulated by soil microorganisms. *Glomus versiforme* (Karsten) Berch spores that failed to germinate in autoclaved, gamma-irradiated, or steamed soils exhibited 65–80% germination in nontreated, nonsterile soils (12). Similarly, *G. mosseae* spores that did not germinate on nutrient broth agar or water agar exhibited 35–40% germination in a pasteurized soil containing living *Coleus* plants (10) and, presumably, some microorganisms. Most recently, specific bacterial and fungal isolates have been identified that stimulate VAM fungal spore germination in vitro. Two rhizosphere bacteria (5) and two unidentified fungal isolates (7) have been reported to stimulate the germination of surface-disinfested *G. mosseae* spores on water agar. Furthermore, one *Pseudomonas* sp., two *Corynebacterium* spp., and two unidentified bacteria isolated from *G. versiforme* spore surfaces were found to stimulate germination of the spores (21). A bacterial spore contaminant of *G. caledonium* promoted germination of spores inhibited by a nonsterile soil extract on water agar (30). Mugnier and Mosse (25) recovered a soil actinomycete, *Streptomyces orientalis* Pittenger and Brigham, as a contaminant of surface-disinfested *G. mosseae* spores on water agar, which stimulated germination of the spores. Additionally, experiments indicated that the stimulatory effect was volatile. This is of potential practical importance as it may facilitate reliable, long-term production of sterile, germinated *G. mosseae* spores.

The experiments described herein were conducted to obtain additional information about the influence of *S. orientalis* on germination of VAM fungal spores. More specifically, the objectives were to characterize the effect of *S. orientalis* on germination of *G. mosseae* and other VAM fungal spores on water agar and to determine whether other *Streptomyces* spp. stimulate VAM fungal spore germination. A preliminary report of this work has been published (31)

MATERIALS AND METHODS

VAM fungi. VAM fungi were increased for 1 yr on Bahia grass (*Paspalum notatum* Flüggé) in greenhouse pot cultures obtained from R. W. Roncadori, Department of Plant Pathology, University of Georgia. Infested soil containing roots and spores was collected and stored in plastic bottles at 2 C unless otherwise noted. Azygospores of *Gigaspora margarita* Becker & Hall and *Scutellispora heterogama* (Nicol. & Gerd.) Walker & Sanders and sporocarps of *G. mosseae* were extracted from soil by combination wet-sieving and decanting (14). Following sucrose

centrifugal-flotation (17), fungal structures were collected by vacuum on Whatman No. 1 filter paper using a Büchner funnel. Chlamydospores of *G. mosseae* were carefully removed from sporocarps under a stereoscopic microscope. Spores of each fungus were individually transferred to previously autoclaved, 1.5-ml microcentrifuge tubes and a filter-sterilized (0.22 μm Millipore filter) solution of 2% (w/v) chloramine T, and 200 ppm streptomycin sulfate (23) was added. Tubes were gently agitated for 30 min at room temperature on an orbital mixer. Spores were subsequently concentrated by centrifugation for 3.5 min at 1,830 g and were rinsed in three changes of sterile distilled water (SDW) for 15 min each as was done with the surface-disinfestant solution. Finally, spores were resuspended in SDW and transferred to a sterile paper towel before plating on agar medium. Petri plates were kept in plastic bags to reduce desiccation and were incubated at 25 C in the dark. A spore was considered germinated when the nascent germ tube reached a length equal to the spore diameter. Spores were immediately excised from the agar and discarded upon observation of fungal or bacterial contamination.

***Streptomyces* cultures.** *Streptomyces* spp. were cultured in 250-ml Erlenmeyer flasks containing 100 ml of sterile mannitol (10 g/L) yeast extract (2 g/L) broth (25). Cultures were grown at room temperature on a wrist-action shaker for 2 days. Microcolonies were recovered from the broth by pouring the culture fluid through a sterile, 7.6-cm-diameter, 38-μm-pore sieve. Microcolonies were rinsed thoroughly on the sieve with sterile distilled water and carefully washed into flasks of sterile, molten 1.5% Noble water agar (WA) (Difco Laboratories, Detroit, MI), which had cooled to 45 C.

S. orientalis was obtained from the American Type Culture Collection (ATCC 21425). *S. avermitilis* Burg et al was obtained from the University of Leeds, England; G. Michaels, Department of Microbiology, University of Georgia, provided the culture of *S. griseus* (Krainsky) Waksman & Henrici. Stock cultures of all species were maintained in mannitol yeast extract broth at 25 C and transferred monthly.

Effect of *S. orientalis* on spore germination. Two initial experiments were conducted to characterize the effect of *S. orientalis* on *G. mosseae* chlamydospore and *G. margarita* and *S. heterogama* azygospore germination. Spores were transferred to 9-cm-diameter plastic petri plates containing 40 ml of 1.5% (w/v) Noble WA or two 20-ml WA layers. Suspended in the lower WA layer of the double-layer WA were living or autoclaved *S. orientalis* microcolonies (25). Ten to 20 VAM fungal spores were transferred per plate, and three to four replicate plates of each type were used. Spores were observed periodically, and frequency of germination was noted.

Volatile effect of *S. orientalis* on spore germination. Two opposite quadrants of sterile, 9-cm-diameter, four-compartment glass petri plates were filled with 4 ml of WA. The remaining two quadrants both received either 4 ml of WA or 4 ml of WA containing *S. orientalis* microcolonies. Six spores were plated on the surface of each of the two opposite quadrants containing WA in each plate. A third treatment consisted of six spores plated directly onto the surface of opposite quadrants filled with WA containing *S. orientalis* microcolonies. All plates were sealed with strips of Parafilm (American Can Company, Greenwich, CT) before incubation at 25 C in the dark. Frequency of spore germination was noted every 14 days for 42 days. The experiment was conducted twice for *G. margarita* and *G. mosseae* and once for *S. heterogama*.

Quantitative effect of *S. orientalis* on spore germination. An experiment was conducted to determine the influence of *S. orientalis* microcolony density in double-layer WA on stimulation of *G. mosseae* spore germination. Plates were prepared as described earlier, except that WA containing three different densities of *S. orientalis* microcolonies in a 10-fold dilution series were used. Eleven or more spores were transferred to five or six replicate double-layer plates for each of the three microcolony densities used or two WA plates. Spores were observed periodically and frequency of germination was noted.

Effect of other *Streptomyces* spp. on spore germination. Two experiments were conducted to determine whether VAM fungal spore germination is affected by other *Streptomyces* spp. Microcolonies of *S. avermitilis, S. griseus*, or *S. orientalis* were collected from broth culture, suspended in WA, and used to form the bottom layer of double-layer WA plates. All plates received a 20-ml overlayer of WA. Two to six replicate plates of each type of double-layer WA plate and WA received 12–19 *G. mosseae* spores. Similar numbers of *S. heterogama* spores were plated on three to seven replicate WA or double-layer WA plates. Spore germination was assessed periodically for 65 days.

Effect of *Streptomyces* spp. on WA pH. The pH of WA and the top WA layer of double layers containing *S. avermitilis, S. griseus, S. orientalis*, or autoclaved *S. orientalis* microcolonies in the bottom WA layer was determined immediately after their preparation and then periodically after incubation at 25 C. The top WA layers of the double-layers were carefully removed from the underlying layer with a spatula. Agar was melted in a microwave oven, diluted with an equal volume of distilled water, and stirred for 60 sec before determination of pH.

Effect of pH on spore germination. Based on the results of experiments described immediately above, two studies were performed to determine the effect of pH on VAM fungal spore germination. Seven to 17 *G. mosseae* or *S. heterogama* spores were plated on each of three to five replicate plates of double-layer WA with *S. orientalis* microcolonies in the bottom layer or WA adjusted to pH 5.0, 6.0, 7.0, or 8.0. The WA was prepared by heating the agar until it dissolved and subsequently adjusting the pH with 1 N NaOH or 1.2 N HCl before autoclaving. Spore germination was assessed every 2–5 days for a total of 65 days.

Statistical analysis. All statistical analyses were performed using the SAS statistical package (28). Germination percentages for treatments at each day of observation were initially subjected to a one-way analysis of variance (ANOVA). Treatment effects determined to be significant by ANOVA at $P = 0.05$ were further separated by multiple means comparisons tests. Fisher's least significant difference test was used when sample sizes were equal and Tukey's studentized range test was employed when unequal sample sizes were present. Changes in pH of agar media over time were characterized by stepwise regression analysis ($P = 0.05$), whereas pH differences between media at individual sampling times were analyzed as was done for spore germination data.

RESULTS

Effect of *S. orientalis* on spore germination. Germination of *G. margarita* azygospores and *G. mosseae* chlamydospores was stimulated by *S. orientalis* (Fig. 1). At 5 days after plating (DAP), frequency of *G. margarita* spore germination on double-layer WA containing *S. orientalis* microcolonies in the bottom layer was significantly greater than on WA and/or double-layer WA prepared with autoclaved *S. orientalis* colonies. For *G. mosseae*, however, spore germination on double-layer WA with *S. orientalis* did not differ significantly from that on WA or double-layer WA with autoclaved *S. orientalis* microcolonies until 7 DAP. No significant differences were found in spore germination frequency on the latter two treatments for either fungus throughout the experiment. Maximum germination frequency for *G. margarita* and *G. mosseae* on double-layer WA with *S. orientalis* was 43 and 74%, respectively.

Streptomyces orientalis consistently stimulated germination of *G. margarita* and *G. mosseae* spores in several experiments. However, the time of onset of germination varied from 5 to 9 DAP for both fungi, and maximum germination frequency ranged from 7 to 43% for *G. margarita* and from 30 to 85% for *G. mosseae*.

Conversely, *S. heterogama* azygospores failed to germinate on double-layer WA with *S. orientalis* but germinated on WA beginning 16 DAP (Fig. 2). Statistically significant differences in spore germination between the two treatments did not occur until 26 DAP. A maximum germination frequency of 32% was observed at 65 DAP. Spores of *S. heterogama* were initially

extracted from infested soil stored at 2 C, but these spores failed to germinate on any treatment. Consequently, *S. heterogama* spores were extracted from 1- to 2-yr-old pot culture soil in the greenhouse immediately before surface disinfestation for these and all subsequent experiments.

Volatile effect of *S. orientalis* on spore germination. Germination of *G. margarita* and *G. mosseae* spores was stimulated by volatile compounds. Comparable numbers of *G. margarita* spores germinated when plated directly onto WA containing *S. orientalis* and when plated on WA in four-compartment petri plates with *S. orientalis* present in WA in separate, opposite quadrants (Table 1). Germination frequency of *G. mosseae* spores was greater when plated on WA with *S. orientalis* in WA in separate quadrants than when plated directly onto WA containing *S. orientalis* at 14 DAP. Differences between the two treatments were not detected at 28 and 42 DAP. Throughout the experiments, spore germination frequency of both VAM fungi was consistently lowest on WA plates.

In contrast, *S. heterogama* spore germination frequency was comparable when spores were placed directly on WA containing *S. orientalis* and on WA at 14 and 28 DAP (Table 1). However, the highest germination frequency occurred when spores were plated on WA and *S. orientalis* was present in WA in separate, opposite quadrants. Observations were not made at 42 DAP for *S. heterogama.*

Quantitative effect of *S. orientalis* on spore germination. The three types of double-layer WA containing different *S. orientalis* microcolony densities will be referred to as 1.0X, 0.1X, and 0.01X. The 1.0X density treatment was comparable to the density used in all experiments described previously.

Stimulation of *G. mosseae* spore germination was not greatly influenced by *S. orientalis* microcolony density. Stimulation of spore germination by *S. orientalis* was observed as early as 5 DAP and was greatest throughout the experiment on the 0.1X density double-layers (Fig. 3). No differences were found in germination frequency between the 1.0X and 0.1X density double-layers. Germination of spores on 0.01X double-layers was less than on 0.1X double-layers until 75 DAP and less than on 1.0X double-layers from 25 to 50 DAP. Spores did not germinate on WA until 45 DAP, but germination frequency increased rapidly thereafter. No differences were detected among treatments by 80 DAP. Maximum germination on 1.0X, 0.1X, and 0.01X double-layers and WA was 76, 85, 71, and 62%, respectively.

Effect of other *Streptomyces* spp. on spore germination. All three *Streptomyces* spp. stimulated germination of *G. mosseae* spores. Germinated spores were first observed on double-layer

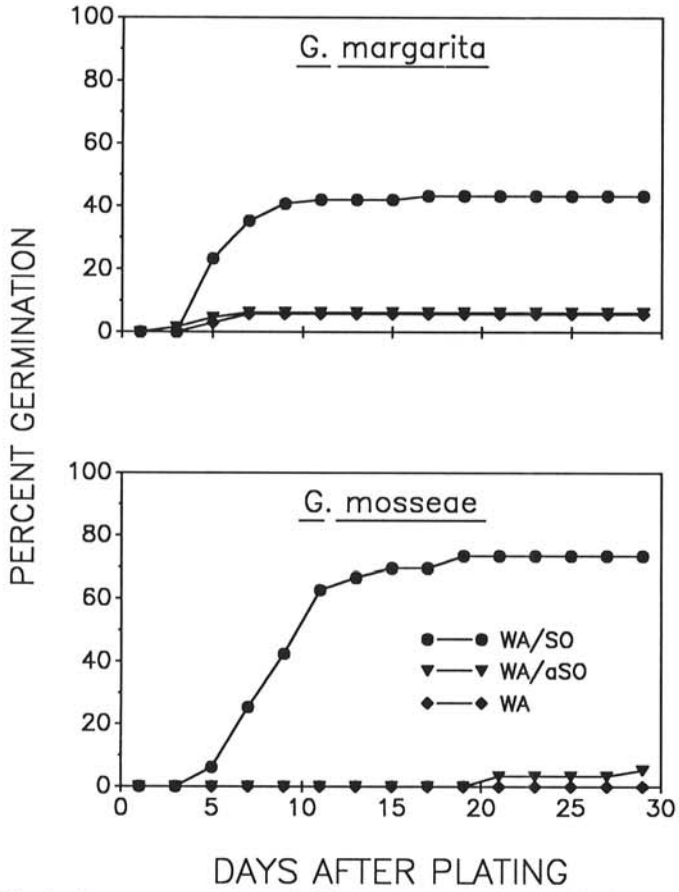

Fig. 1. *Gigaspora margarita* and *Glomus mosseae* spore germination on 1.5% Noble water agar (WA) and double-layer WA containing *Streptomyces orientalis* microcolonies (WA/SO) and autoclaved *S. orientalis* microcolonies (WA/aSO) in the bottom WA layer.

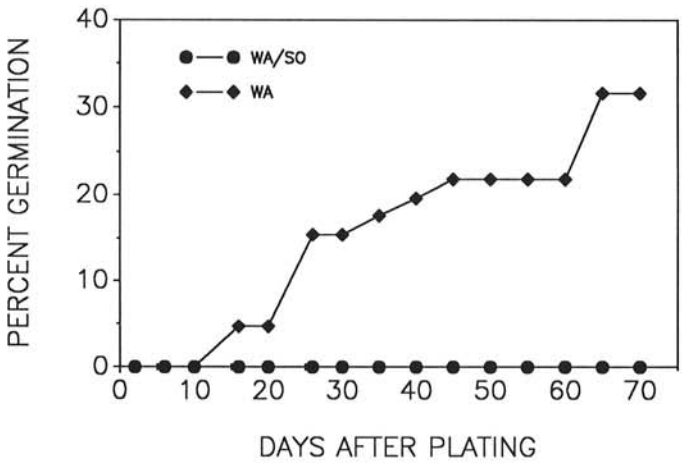

Fig. 2. Germination of *Scutellispora heterogama* spores on 1.5% Noble water agar (WA) and double-layer WA containing *Streptomyces orientalis* microcolonies in the bottom WA layer (WA/SO).

TABLE 1. Germination of vesicular-arbuscular mycorrhizal fungal spores in four-compartment petri plates containing 1.5% Noble water agar (WA) with and without *Streptomyces orientalis* microcolonies

	Percent germination[a]					
	Day 14			Day 28		
Treatment[b]	*Gigaspora margarita*	*Glomus mosseae*	*Scutellispora heterogama*	*Gigaspora margarita*	*Glomus mosseae*	*Scutellispora heterogama*
Direct	27.9 a[c]	50.0 b	43.9 b	30.8 a	64.3 a	45.2 b
Indirect	23.6 a	77.5 a	81.4 a	26.4 a	82.6 a	81.4 a
WA	2.6 b	0.0 c	53.5 b	2.6 b	0.0 b	56.3 b

[a] Percent germination values are means of seven direct and indirect and eight WA plates for *G. margarita*, three direct and indirect and four WA plates for *G. mosseae*, and six plates of all treatments for *S. heterogama*.

[b] Direct = spores plated directly onto WA containing *S. orientalis* microcolonies; Indirect = spores plated onto WA in plates containing *S. orientalis* microcolonies in WA in separate, opposite quadrants; WA = spores plated onto WA in plates containing WA without *S. orientalis* microcolonies in all quadrants.

[c] Means within the same column followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference test for *S. heterogama* and Tukey's studentized range test for *G. margarita* and *G. mosseae*.

WA containing *S. griseus* at 5 DAP (Fig. 4). No statistical differences were found in germination frequency between double-layers containing *S. avermitilis* and *S. griseus* throughout the experiment. Germination frequency was less on double-layer WA containing *S. orientalis* than *S. avermitilis* until 21 DAP and less than on double-layer WA with *S. griseus* until 25 DAP. No differences were detected between the double-layers containing the *Streptomyces* colonies after this time. Germination frequency increased on double-layers containing *S. orientalis* beginning 9 DAP but was not significantly different from WA until 21 DAP. Spores did not germinate on WA through 65 DAP. Maximum germination on double-layer WA with *S. avermitilis, S. griseus*, and *S. orientalis* was 74, 77, and 57%, respectively.

Germination of *S. heterogama* spores was suppressed by *S. avermitilis* and *S. orientalis*. Germinated spores were first observed on WA 6 DAP, and germination frequency increased through 65 DAP to a maximum of 30% (Fig. 4). Spores first germinated on double-layer WA with *S. orientalis* at 10 DAP but never germinated on double-layer WA containing *S. avermitilis*. Germination frequency was statistically greater on WA than on double-layer WA with *S. avermitilis* or *S. orientalis* beginning 26 DAP. Maximum germination frequency on double-layer WA containing *S. orientalis* was 3% and did not differ from double-layer WA with *S. avermitilis* throughout the experiment.

Effect of *Streptomyces* spp. on WA pH. All *Streptomyces* spp. increased the pH of WA overlayers in double-layer WA from the initial pH of 5.2 (Table 2). The pH of WA overlayers of double-layers containing *S. avermitilis* and *S. griseus* was significantly higher than double-layers containing autoclaved *S. orientalis* microcolonies or WA after 4 h, whereas an increase in pH for double-layer WA with *S. orientalis* was not detected until day 1. The pH of WA and double-layer WA with autoclaved *S. orientalis* was the same throughout the experiment and remained unchanged. The increase in pH of double-layer WA with *S. avermitilis* and *S. orientalis* over time was cubic, whereas pH of double-layer WA with *S. griseus* increased linearly. No differences were detected in pH among double-layers containing *S. avermitilis, S. griseus*, and *S. orientalis* at the end of the experiment.

Effect of pH on spore germination. Spores of *G. mosseae* failed to germinate on WA regardless of the pH (Fig. 5). Germinated spores were first observed on double-layer WA containing *S. orientalis* at 8 DAP and germination frequency increased through 45 DAP. Germination frequency on these plates was significantly different from WA of all pH beginning 16 DAP and reached a maximum of 56%.

Germination of *S. heterogama* spores was affected by WA pH and was first observed 6 DAP on pH 7 and pH 8 WA (Fig. 5). Germination frequency was inversely related to WA pH. However,

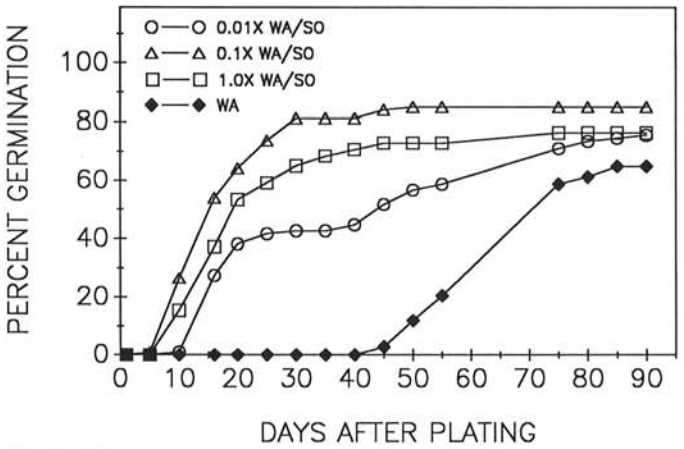

Fig. 3. *Glomus mosseae* spore germination on 1.5% Noble water agar (WA) and double-layer WA (WA/SO) containing one of three densities of *Streptomyces orientalis* microcolonies in the bottom WA layer. Densities are tenfold dilutions and treatments are designated 1.0X, 0.1X, and 0.01X WA/SO.

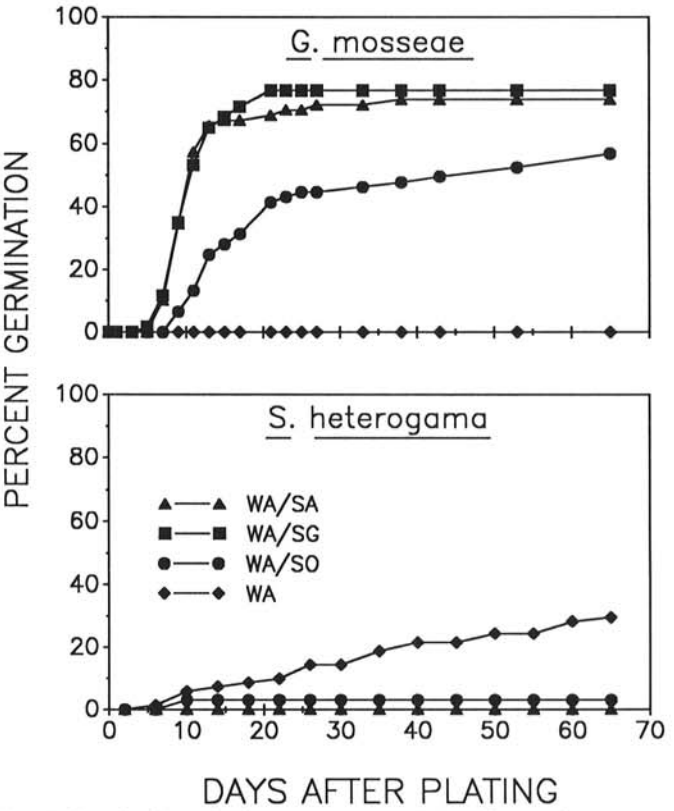

Fig. 4. Germination of *Glomus mosseae* and *Scutellispora heterogama* spores on 1.5% Noble water agar (WA) and double-layer WA containing microcolonies of *Streptomyces avermitilis* (WA/SA), *S. griseus* (WA/SG), and *S. orientalis* (WA/SO) in the bottom WA layer. *Scutellispora heterogama* spores were not plated on WA/SG.

TABLE 2. Effect of *Streptomyces* spp. on pH of 1.5% Noble water agar overlayers in double-layer plates

Treatment[b]	pH[a]								Regression[c]
	4 h	day 1	day 3	day 5	day 9	day 13	day 17	day 21	
S. avermitilis	5.8 a[d]	6.7 a	7.2 a	7.8 a	7.9 a	7.4 a	7.6 a	7.6 a	cubic ($R^2 = 0.86$)
S. griseus	6.0 a	6.5 a	6.6 b	6.5 c	6.9 b	7.3 a	7.4 a	7.6 a	linear ($R^2 = 0.77$)
S. orientalis	5.5 b	5.7 b	6.6 b	6.8 b	7.0 b	6.8 b	6.8 b	7.0 a	cubic ($R^2 = 0.95$)
Autoclaved *S. orientalis*	5.3 b	5.3 c	5.3 c	5.3 d	5.2 c	5.4 c	5.2 c	5.2 b	NS[e]
WA	5.2 b	5.4 c	5.2 c	5.2 d	5.2 c	5.3 c	5.2 c	5.2 b	NS

[a] All pH values are means from four plates per treatment.
[b] Double-layer water agar plates containing *S. avermitilis, S. griseus,* or *S. orientalis* microcolonies, autoclaved *S. orientalis* microcolonies, or no microcolonies (WA) in the bottom layers.
[c] Results of stepwise regression analysis of pH vs. incubation time in days ($P < 0.05$).
[d] Means within columns followed by the same letter are not significantly different according to Fisher's least-significant-difference test ($P = 0.05$).
[e] NS = Not significant.

no statistical differences were found in spore germination frequency between double-layer WA with *S. orientalis* and pH 8 WA or among pH 5, 6, and 7 WA throughout the experiment. Frequency of spore germination on pH 7 WA was not significantly different from germination on pH 8 WA or double-layer WA with *S. orientalis* from 12 to 20 DAP. Germination frequency at 50 DAP on pH 5, 6, 7, and 8 WA was 85, 77, 73, and 47%, respectively. Maximum germination on double-layer WA with *S. orientalis* was 49%.

DISCUSSION

Germination of *G. margarita* azygospores and *G. mosseae* chlamydospores was stimulated by *S. orientalis*. Furthermore, at least one compound responsible for the stimulation was volatile since spore germination increased in the presence of *S. orientalis*, even when the actinomycete was physically separated from WA on which the spores were plated. The stimulatory effect of *S. orientalis* on spore germination of *G. mosseae* was reported in 1987 (25) but had not been previously documented for *G. margarita*. Additionally, two other species of *Streptomyces* also stimulated *G. mosseae* spore germination in our experiments. The nonspecific nature of this phenomenon is, perhaps, an indication that germination of VAM fungal spores in natural soils is influenced by the activity of many *Streptomyces* spp. or other actinomycetes present.

The volatile stimulus produced by *S. orientalis* that elicited spore germination may be a novel gaseous compound not normally present in ambient air. Alternatively, growth of *S. orientalis* may change the concentration of one or more gases present in air resulting in stimulation of spore germination. Le Tacon et al (19) found that germination of axenic *G. mosseae* spores was affected by oxygen and carbon dioxide tensions.

Although *S. orientalis* consistently stimulated germination of *G. margarita* and *G. mosseae* spores, the degree to which and rate at which germination occurred varied. Differences in *S. orientalis* microcolony density in double-layer WA probably did not contribute to the variation since hundredfold differences in microcolony density were needed to affect the degree and rate of stimulation of *G. mosseae* spore germination. Variability in spore germination frequency possibly resulted from osmotic damage due to sucrose centrifugal-flotation during spore collection or the effect of storage time and conditions on the spores. Influence of the latter factors on VAM spore germination is documented (13,20,29). The infested soils from which *G. margarita* and *G. mosseae* spores were extracted for these experiments had been stored at 2 C for varying periods of time. Furthermore, no measures were taken to standardize or control moisture content of the soil during storage. Consequently, the overall germination potential of different batches of spores may have been affected by the variable storage conditions.

Germination of *G. mosseae* spores on WA was negligible in all experiments through 45 DAP and was not affected by pH. Poor germination of *G. mosseae* spores on WA has been previously reported (10,11,25,32), although moderate to high levels of germination (4,5,7,15) have also been observed. The basis for these discrepancies is unknown.

Germination of *S. heterogama* spores was not stimulated by the *Streptomyces* spp. examined, perhaps because of the increase in pH of WA caused by the underlying actinomycete colonies. This hypothesis is supported by the observed inverse relationship between WA pH and *S. heterogama* spore germination frequency. Results of the experiment in which four-compartment petri plates were used indicated that *S. heterogama* spore germination was stimulated by *S. orientalis* when physically separated from the WA containing the organism. Germination of these spores was apparently inhibited by some factor associated with the growth of *S. orientalis* in WA.

It is unknown whether the stimulation of spore germination by *Streptomyces* spp. is a natural phenomenon or an artifact of these in vitro experiments. However, microbial stimulation of VAM spore germination in soil has been reported (10,12).

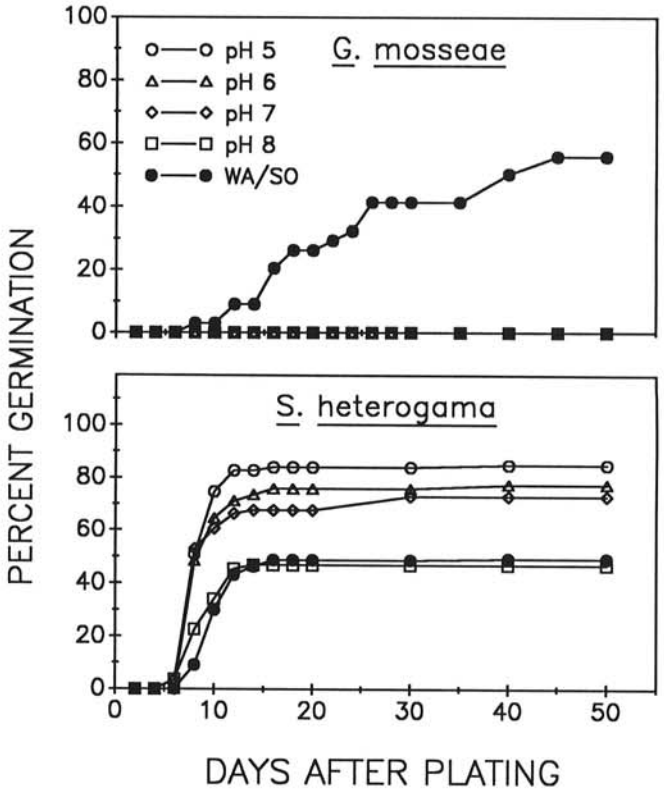

Fig. 5. *Glomus mosseae* and *Scutellispora heterogama* spore germination on 1.5% Noble water agar (WA) adjusted to pH 5, 6, 7, and 8 and double-layer WA containing *Streptomyces orientalis* microcolonies in the bottom WA layer (WA/SO).

Furthermore, actinomycetes were observed on the surface of spores (9), and *Streptomyces* spp. were isolated from VAM fungal spores (1,3). It is also uncertain what effect stimulation of spore germination would have on the establishment of vesicular-arbuscular mycorrhizae in roots of higher plants. Soil microorganisms decreased VAM fungal colonization, sporulation, and the stimulation of host plant growth (16,18,27). Conversely, VAM fungal colonization was increased by a suspension of soil microorganisms (6), two specific rhizosphere bacteria (5), and several chitin-decomposing actinomycetes, including *Streptomyces* spp. (2). If microorganisms such as the *Streptomyces* spp. examined in these experiments are shown to promote VAM fungal spore germination and subsequent colonization in natural environments, they could potentially be used for increasing the effectiveness of commercial VAM fungal inocula.

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