Effect of liming on spore germination, germ tube growth and root colonization by vesicular-arbuscular mycorrhizal fungi*

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Summary The effect of soil acidity on spore germination, germ tube growth and root colonization of vesicular-arbuscular mycorrhizal (VAM) fungi was examined using a Florida Ultisol. Soil samples were treated with 0, 4, 8 and 12 meq Ca/MgCO3/100 g soil and each lime level received 0, 240, and 720 ppm P as superphosphate. Corn (Zea mays L.) was planted in the soil treatments, inoculated with either Glomus mosseae or Gigaspora margarita spores and grown for 31 days. Acid soil inhibits mycorrhizal formation by G. mosseae through its strong fungistatic effect against the spores. The dolomitic lime increased mycorrhizal formation by both fungal species. G. margarita is much less sensitive to acidic conditions than G. mosseae.

Introduction

A possible alternative mechanism for maximizing fertilizer efficiency is via inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi. They enhance nutrient uptake, and consequently plant growth, through an extensive network of external mycelium which acts as an extension of the root absorption system17. This is particularly important in infertile soils, commonly found in tropical regions, and appears to be a promising technology for subsistence farmers23. If economical techniques for inoculation or manipulation of the native population of VAM fungi are to be developed, the adaptability of the fungi to edaphic factors must be considered. Soil acidity is of immediate concern since it is known to affect spore distribution, root colonization and mycorrhizal efficiency4,18,19. However, it is not clear if this results from direct activity of the ionic concentration of H+ and/or from changes in the chemical properties of the soil. Hubbell12 suggests that liming may influence the development of mycorrhizal associations either by decreasing the growth of fungal propagules in the rhizosphere or by decreasing fungal colonization of root tissue.

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Germination of VAM fungal spores on either agar media or soil is affected by the pH. However, soil acidity is not an independent factor; pH alone may have little significance in understanding fungal spore germination and root colonization by these fungi. We have recently found significant interactions between pH and medium composition for spore germination "in vitro". This suggests that the effect of soil acidity on VAM fungi may be the result of changes in solubility of nutrients and toxic elements in the soil environment, rather than pH "per se".

This study attempts to evaluate the effect of soil acidity on spore germination, germ tube growth and colonization of corn root by two VAM fungi.

Materials and methods

Samples of a virgin Dothan fine sandy loam (thermic Plinthic Paleudult) soil were collected at 0–20 cm depth from an area under a 20-year-old stand of slash pine in Escambia County, Florida. The samples received the equivalent of 0, 4, 8 and 12 meq Ca/MgCO₃/100 g of soil (as dolomitic limestone containing 54% CaCO₃ and 43% MgCO₃). After incubation for 3 months with the water content maintained at 22% by weight, 0, 240, and 720 ppm of P as superphosphate (46% P₂O₅) was applied simultaneously with 200 ppm of K as KCl and 20 ppm of trace elements (frit) as FTE 503 (18% Fe, 7.5% Mn, 7.0% Zn, 3% B and 0.2% Mo). The samples were again incubated for 3 months, then air dried and stored in plastic bags until used.

For the greenhouse experiments, 250 g of soil was packed into styrofoam cups. Fifty spores per cup of either Glomus mosseae or Gigaspora margarita were applied 3 cm below the soil surface, and 25% w/w water was added. This was a factorial experiment composed of 4 lime treatments × 3 P levels × 3 VAM treatments × 3 replications. Three imbibed seeds of corn (Zea mays L. var. Dekalb) were planted per pot and thinned to one plant five days after emergence. After growing for 31 days under greenhouse conditions, plants were harvested and individual root samples were cleared and stained for root colonization assessment using the grid intersect method.

At the same time, soil samples with no applied P and different limestone treatments were brought to the laboratory for spore germination studies. Twenty spores of either G. mosseae or G. margarita were placed between two Gelman membrane filters (.45 μm) and incubated between two 50 g layers of soil in 100 × 15 mm Petri dishes. Each limestone treatment was triplicated. Washed river sand was used as control. Soil moisture was adjusted to 25% w/w, the plates were wrapped with aluminium foil and incubated at 28°C for 20 days. After incubation, filters with spores were removed from the plates and flooded with either trypan blue or acid fuchsin (both 0.01%) staining solution for 5 min. Following staining, spores were observed under a dissecting microscope at ×12 or 25 and spore germination and germ tube growth rate were recorded according to Siqueira et al.25.

Water extracts from the different soil samples were obtained using 100 g/100 ml of deionized water, shaken for 48 hours and prepared according to Ko and Hora. These were incorporated in agar plates for studies of their effects on spore germination and growth features. Experimental details are given by Siqueira26 and Siqueira et al.25 but only data for germination are included here (Fig. 2).