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Tissue magnesium and calcium affect arbuscular mycorrhiza development and fungal reproduction

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Abstract Applications of high levels of $MgSO_4$ resulted in reduced root colonization and sporulation by *Glomus* sp. (INVAM isolate FL329) with sweet potato and onion in aeroponic and sand culture, respectively. Onion shoot-Mg concentrations were elevated when a nutrient solution containing 2.6 or 11.7 mM $MgSO_4$ was applied. Magnesium application depressed tissue-Ca levels. With lower Ca in the tissue, colonization was reduced from > 30% of root length to < 10%, and sporulation from > 1200 to ca. 200 spores per plant, 10 weeks after transplantation and the start of nutrient application. These effects on colonization and sporulation were independent of changes in tissue-P concentration. High Mg/low Ca tissue concentrations induced premature root senescence, which may have disrupted the mycorrhizal association. Our results confirm the importance of Ca for the maintenance of a functioning mycorrhiza.

Key words Colonization · Sporulation · Nutrient effects · Soilless culture

Introduction

Soil bases such as Mg and Ca have a role in root colonization and sporulation of arbuscular mycorrhizal (AM) fungi. The importance of Ca was first reported by Hepper and O'Shea (1984) who found Ca supply was critical for colonization of lettuce by both *Glomus caledonium* (Nicol. & Gerd.) Gerdmann & Trappe and *G. mosseae* (Nicol. & Gerd.) Gerdmann & Trappe. Anderson and Liberta (1992) found that base-treated plants had higher colonization, but less sporulation than those without such amendments. These findings were in agreement with previous work showing that soil Ca was a good predictor of sporulation (Anderson et al. 1984). Likewise Habte and Soedarjo (1995) found higher colonization of *Leucaena leucocephala* by *G. aggregatum* (Schenck & Smith) Koske with addition of moderate levels of Ca from either lime or gypsum in an acid soil. Gryndler et al. (1992) reported that AM colonization and aboveground biomass of maize were enhanced by added Mg. The highest levels of colonization and biomass were achieved with application of 11.68 mM $MgSO_4$.

We have grown *Glomus* spp. in aeroponic culture by providing a balance of conditions promoting the spread and reproduction of the AM fungus (Hung and Sylvia 1988). With reports of enhanced colonization from elevated Mg (Gryndler et al. 1992) and the linkage between colonization and sporulation (Simpson and Daft 1990), we sought to more thoroughly test the effect of increased Mg in inoculum production systems. The objective of our study was to evaluate the effect of applied $MgSO_4$ on the spread of colonization and subsequent sporulation of an AM fungus in both aeroponic and sand culture systems.

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Materials and methods

Aeroponic experiment

Spores of *Glomus* sp. (INVAM isolate FL329) were collected from a 6-month-old pot culture by sieving and decanting followed by surface disinfestation with 2% chloramine T and 0.02% streptomycin sulfate (Mosse 1962). After filling each 2-l plastic pot half-full with new horticultural vermiculite, 300–500 disinfested spores were mixed in a layer approximately 2 cm deep and vermiculite was added to within 1 cm of the pot rim. Two-node cuttings

of sweet potato (*Ipomoea batatas* (L.) Lam. cv. 'White Star') were washed thoroughly with insecticidal soap and placed into the vermiculite so that the upper nodes were exposed. Each pot was watered thoroughly and as necessary to maintain well-watered conditions. The culture plants were grown in a high-intensity-discharge (HID) growth chamber with a maximum photosynthetic photon fluence rate (PPFR) of 900 μmol m^{-2} s^{-1}, 29/23 °C day/night temperatures, 65/80% relative humidity and a 16-h day length. After 6 weeks, the vermiculite was removed by shaking and washing the roots. Colonization was verified by the method of Ames et al. (1982). Roots were kept moist until they were placed into an aeroponic chamber where a dilute nutrient solution was sprayed intermittently (7 s for every 90 s). The dilute nutrient solution contained 1.5 mM KNO_3, 1.5 mM $Ca(NO_3)_2$, 0.6 mM $MgSO_4$, 45 μM NaCl, 30 μM NaFeEDTA, 13.88 μM H_3BO_3, 3 μM KH_2PO_4, 2.74 μM $MnCl_2 \cdot 4H_2O$, 0.96 μM $CuSO_4 \cdot 5H_2O$, 0.23 μM $ZnSO_4 \cdot 7H_2O$, and 0.02 μM $Na_2MoO_4 \cdot 2H_2O$. This solution was adjusted to an initial pH of 6.5 followed by weekly readjustment to the same pH. The average maximal and minimal greenhouse temperatures during the 6-week stabilizing period were 36 and 25 °C, respectively. Average maximal PPFR was 1447 μM m^{-2} s^{-1}. After this initial 6-week period, 36 plants were selected for uniformity and transferred to continuous misting aeroponic chambers to create a split-plot experimental design. The nutrient solution was as above but with 0.6 mM or 11.7 mM $MgSO_4$. These cultures were maintained as previously described (Jarstfer and Sylvia 1995) with all nutrients, including $MgSO_4$, replenished biweekly and the pH maintained as above. The plants were grown from 8 October 1993 to 7 January 1994 in a nonshaded greenhouse with average maximal and minimal temperatures of 31 °C and 23 °C, respectively. Average maximal PPFR was 966 μmol m^{-2} s^{-1} plus supplemental light from 1000 W metal halide lamps which extended the daylength to 14 h at a PPFR of 800 μmol m^{-2} s^{-1} at plant height. At 0, 2, and 13 weeks after transferring to the continuous spray chambers, 3 randomly selected plants were removed for analysis. Spores were collected on a 45-μm sieve after spraying roots with high pressure water over a 425-μm sieve. The roots of each plant were cut into 3-cm-long sections, cleared and stained after Phillips and Hayman (1970), and assessed for percentage colonization by the gridline-intersect method (Giovannetti and Mosse 1980). A general linear model (SAS Institute 1989) was applied to the data to determine the significance of main effects and interactions on sporulation and percentage colonization. This was followed by *t*–tests to separate the means at each time.

Sand culture experiments

Colonized onion single quotes (*Allium cepa* L. cv. 'Evergreen Long White Bunching') transplants were produced from seed by inoculating sand:vermiculite (1:1 v/v) with surface-disinfested spores of *Glomus* sp. (INVAM isolate FL329). Seedlings were grown in the HID chamber under the conditions stated above. Beginning 4 weeks after seeding, 1/10 strength Hoagland's solution (Hoagland and Arnon 1937) was applied to the seedlings on a weekly basis. At 16 weeks from seeding, transplants were separated and washed free of the sand-vermiculite substrate and 90 uniform plants were transferred to autoclaved, acid-washed sand (0.5–1.5 mm diameter) in pinecells (66 ml, Ray Leach Cone-Tainers, Stuewe and Sons Inc., Corvallis, Ore., USA). After preparation, the pH of the sand was 6.5 and, based on Mehlich-I extraction (Hanlon et al. 1990) contained 123 μg g^{-1} Mg, 20.5 μg g^{-1} Ca, and 15.3 μg g^{-1} P.

Plants were grown in a nonshaded greenhouse from 12 February 1994 to 5 May 1994. The average maximal and minimal greenhouse temperatures during the first experiment were 33 °C and 25 °C respectively, and the average maximal PPFR was 1363 μmol m^{-2} s^{-1}. The corresponding environmental parameters for the replicate experiment, started 1 week later, were 33/25 °C and a PPFR of 1393 μmol m^{-2} s^{-1}, respectively. Treatments were arranged in a completely randomized design and plants were grown in an area of less than 0.25 m^2.

Dilute nutrient solutions were prepared as above with low (base line), medium, and high levels of $MgSO_4$ containing 0.6, 2.6 and 11.7 mM $MgSO_4$, respectively. The pH of each solution was adjusted to 6.5 with 10% KOH or 20% H_2SO_4. Plants in each $MgSO_4$ treatment were watered daily to above field capacity with the corresponding solution for 6 days and leached with deionized water on the seventh day.

Ten plants were harvested at transplanting (time 0). Five plants from each treatment were harvested at 2, 4, 6, 8, and 10 weeks after transplanting. The sand, roots and spores were separated by washing vigorously over 425- and 45-μm sieves. Roots on the 425-μm sieve were sprayed vigorously to remove any adhering spores. Roots were cleared and stained, and total and colonized root length assessed by the gridline-intersect method. All spores on the 45-μm sieve were counted. Shoots were dried at 70 °C, weighed, ground to pass a 20-mesh sieve, ashed and analyzed using inductively coupled argon plasma spectroscopy (Model 61E, Thermo-Jarrell Ash Corp., Franklin, Md., USA). A general linear model (SAS Institute 1989) was applied to the data to test for the main effects (time and magnesium sulfate treatment) and their interactions on all response variables. There was no significant difference between the two experiments and so the data were combined for analysis.

Results

Aeroponic experiment

Although sporulation increased with time ($P < 0.0001$), it was depressed by addition of Mg to the nutrient solution ($P < 0.01$). With the higher level of $MgSO_4$, the average number of spores per plant was fourfold lower than the baseline level (Table 1). Sporulation was limited to the original root system with 11.7 mM $MgSO_4$ and most of the root mass was decaying or senescent. Upon microscopic examination (×50), senescent tissue appeared translucent, with epidermal and stele tissue alone remaining intact. No signs or symptoms of disease were present. Plants grown with 0.6 mM $MgSO_4$ had sporulation along the entire length of the root mass and roots were healthy with few senescent parts. The percentage of root length colonized was not significantly affected by either time or applied $MgSO_4$. Mean root colonization was very low, probably because the aeroponic cultures were grown

Table 1 Effect of $MgSO_4$ concentration on arbuscular mycorrhizal colonization and sporulation associated with sweet potato in aeroponic culture. The data are means ±SE, $n=3$. Means followed by the same letter at each time point are not significantly different ($P=0.05$)

Time in experimental conditions (weeks)	$MgSO_4$ (mM)	Colonized root length (%)	Sporulation (spores per plant)
0	0.6	0.7 ± 0.4	400 ± 167
2	0.6	2.4 ± 1.1 A	496 ± 164 A
	11.7	2.0 ± 0.9 A	445 ± 198 A
13	0.6	1.6 ± 0.4 A	3738 ± 457 A
	11.7	1.0 ± 0.3 A	873 ± 314 B

Table 2 Analysis of variance for the effects of applied $MgSO_4$ and time on the arbuscular mycorrhiza of onion and *Glomus* sp. (INVAM isolate FL329) in sand culture (probability values of the *F*-test)

	Shoot dry wt.[a]	Root length[a]	Colonized root length (%)[b]	Sporulation[a]	Tissue P	Tissue Mg	Tissue Ca
Time	0.001	0.0375	0.0001	0.0001	0.0001	0.0001	0.0001
Magnesium	NS	NS	0.0001	0.0001	NS	0.0001	0.0001
Interaction	NS	NS	0.0095	0.0019	NS	0.0001	NS
r	0.73	0.50	0.69	0.74	0.94	0.89	0.82
c.v.	33.4	7.3	44.5	55.6	22.6	25.6	25.9

[a] Data log (x+1) transformed prior to statistical analysis
[b] Data angular transformed prior to statistical analysis

during the fall of the year and colonization for the entire root system was averaged.

Sand culture experiments

Onion growth was not affected by the level of applied $MgSO_4$, with shoot mass and root length both increasing uniformly and significantly with time (Table 2) to a maximum 6 weeks after transplanting (Fig. 1A, B). However, applied $MgSO_4$ did significantly affect development of the mycorrhiza (Table 2). The percentage of root colonization declined during the first 4 weeks after transplantation (Fig. 2A). After that time, plants given 0.6 mM $MgSO_4$ had significantly higher levels of colonization than plants given 2.6 or 11.7 mM $MgSO_4$. By 10 weeks after transplanting, mycorrhizal colonization of plants given 11.7 mM $MgSO_4$ was substantially lower than for those given 0.6 or 2.6 mM $MgSO_4$. Reproduction of the mycorrhizal fungus was similarly affected by $MgSO_4$ application. Significantly higher sporulation was associated with plants given 0.6 or 2.6 mM $MgSO_4$ than at the higher level (Fig. 2B). Variation in the number of spores produced per plant was related to changes in percent colonization ($P < 0.0001$; $r = 0.74$) from 6 to 10 weeks after transplanting, but was not related to shoot mass ($R = 0.31$) or total root length ($R = 0.68$). Applying different levels of $MgSO_4$ in the nutrient solution significantly altered the tissue Mg content of the shoots (Table 2); higher concentrations in the nutrient solution resulted in higher concentrations of Mg in the

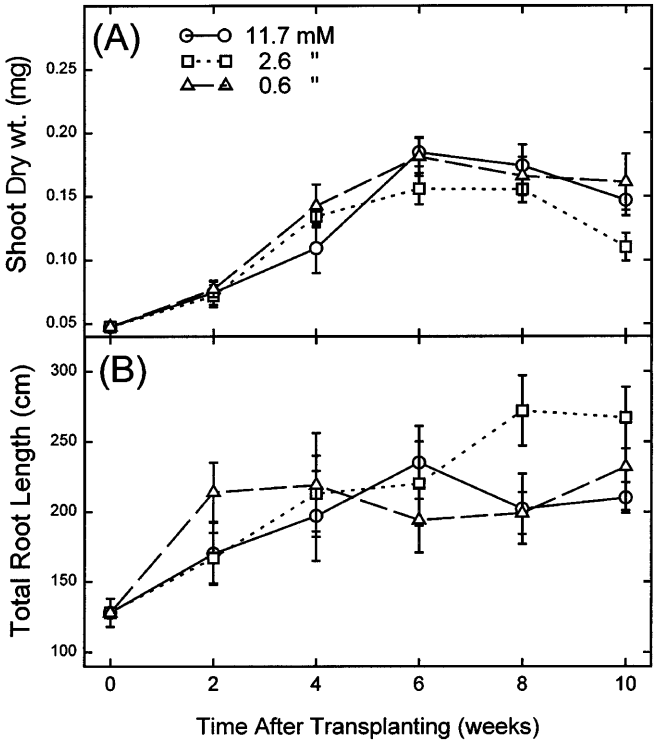

Fig. 1 Growth of onion in response to three levels of applied $MgSO_4$ over a 10-week period following transplantation into sand. **A** Shoot dry wt. per plant; **B** total root length per plant. The data are the means of 5 plants ± SEM

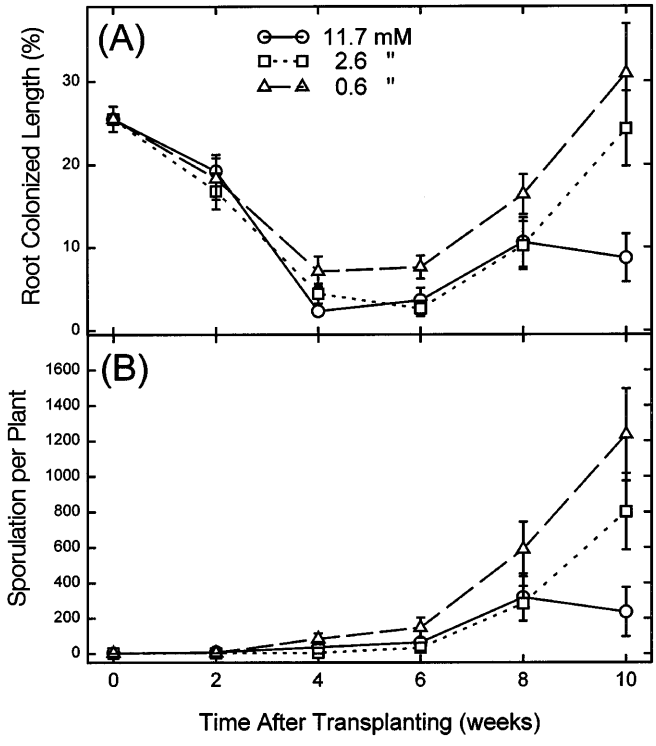

Fig. 2 Development of *Glomus* sp. on onion in response to three levels of applied $MgSO_4$ over a 10-week period following transplantation into sand. **A** Root length colonized; **B** sporulation. The data are the means of 5 plants ± SEM

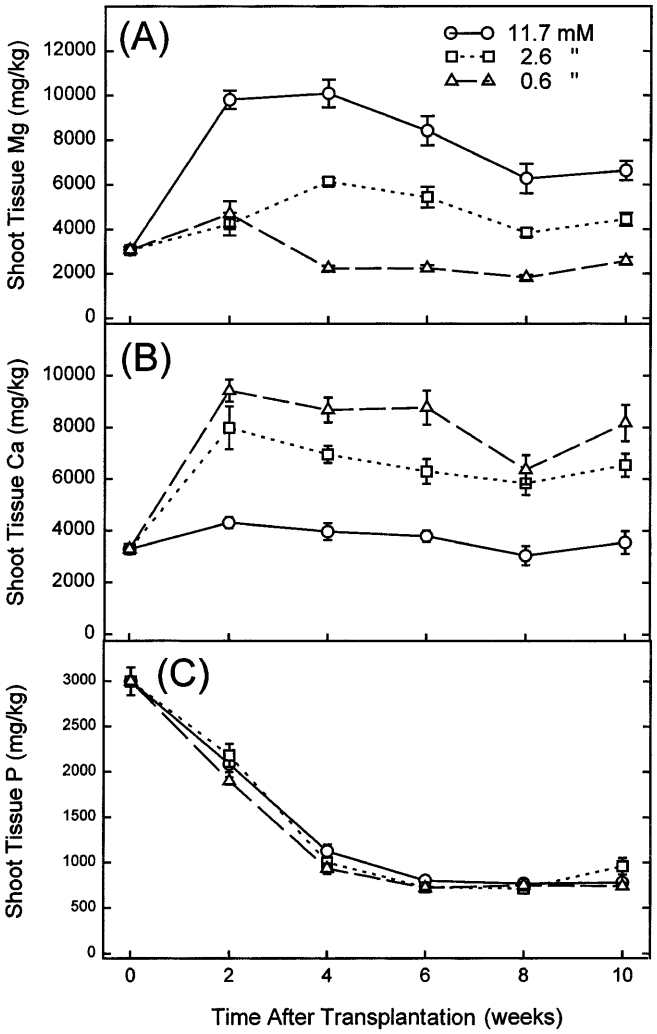

Fig. 3 Onion shoot-tissue concentrations of Mg, Ca, and P following application of three levels of $MgSO_4$ over a 10-week period following transplantation into sand. The data are the means of 5 plants ± SEM

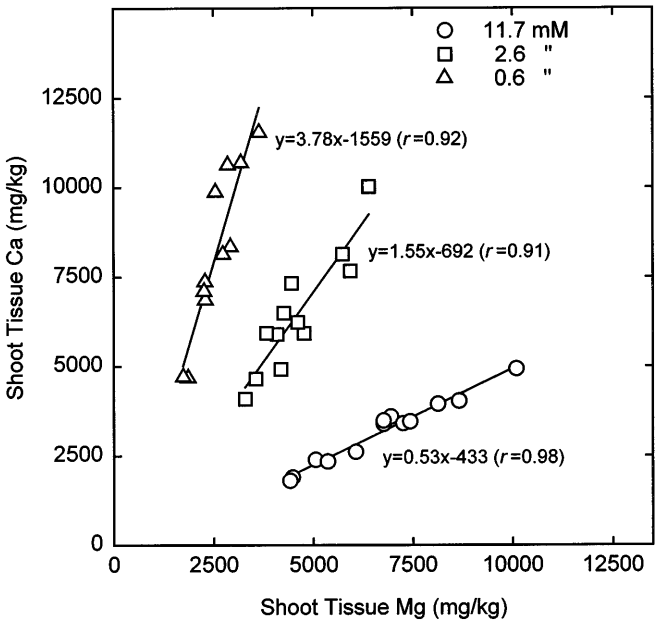

Fig. 4 The relationship between onion shoot-tissue Ca and Mg concentrations 10 weeks after transplantation at different concentrations of applied $MgSO_4$

shoot tissue (Fig. 3A). This effect was clearly evident from 4 weeks after transplanting and tissue concentrations of Mg were maintained to the final harvest. Calcium utilization was also significantly affected by the level of applied $MgSO_4$ (Table 2); however, the effect was the inverse of the effect on tissue Mg concentration (Fig. 3B). Neither Mg nor Ca approached previously published deficiency concentration levels of 340 mg kg^{-1} and 1800 mg kg^{-1}, respectively (Bender 1993). Magnesium concentrations in shoots of plants given 0.6 mM $MgSO_4$ and Ca concentrations in shoots of plants given 11.7 mM $MgSO_4$ fell below previously published sufficiency levels for onion of 3700 mg kg^{-1} and 5200 mg kg^{-1}, respectively (Bender 1993). Correlations existed between tissue Ca and tissue Mg concentrations for each level of applied $MgSO_4$ 10 weeks after transplanting (Fig. 4). Levels of P in shoot tissue declined with time (Fig. 3C), but were not affected by the applied $MgSO_4$ levels (Table 2).

Discussion

Our data are in agreement with the horticultural and agronomic literature stating that Mg fertilization usually depresses Ca uptake (Mengel and Kirkby 1982). Contrary to the results of Gryndler et al. (1992), we found elevated levels of $MgSO_4$ did not increase biomass or AM colonization in either onion or sweet potato. In fact, our results indicate a negative effect on mycorrhizal development independent of P nutrition. Given that the experimental plants were colonized prior to exposure to elevated $MgSO_4$, these studies provide information on the spread of colonization and subsequent reproduction of AM fungi, but do not account for possible effects of Mg on spore germination and initial colonization events.

The inverse relationship between tissue concentrations of Mg and Ca shows that their physiology is closely linked. However, the development of a negative effect on the AM fungus implies a function for Ca ions that cannot be replaced by Mg ions. Calcium is a required component of the middle lamellae and is essential for intracellular membrane transport (Alberts et al. 1994). Both Ca and Mg function to stabilize membranes by bridging phosphate and carboxylate groups of the phospholipids and proteins at the membrane surfaces (Legge et al. 1982). It is then not surprising that Ca deficiency results in cellular disorder and disintegration typical of senescent tissue (Kirkby and Pilbeam 1984), including both lack of ion selectivity and leakage of endogenous respiratory substrates (Bangerth et al. 1972).

Loss of compartmentation and senescence of tissue would affect the immediate functioning of the mycorrhiza on a cellular level and the long-term spread of colonization. The immediate effect of losing ion selectivity and leaking of substrates would be a loss of the carbon source for the AM fungus as well as exposure of the arbuscule to potentially damaging compounds such as peroxidases. Given that Ca is needed for cell growth and is not remobilized, root tissue concentrations falling below a critical level in the long term could slow or stop colonization for lack of suitable root substrate (i.e., new cortical cells) (Marschner and Richter 1974). Simpson and Daft (1990) showed that no new spores were produced when maize reached physiological maturity and colonized root length ceased to increase, possibly indicating the close relationship between sporulation and colonization of new cortical tissue. The importance of new cortical cells to colonize is also supported by what is known of carbon allocation and transfer to AM fungi and the central importance of the arbuscule in this process (Smith and Smith 1990). The effect of a Ca deficiency on the formation of new cortical cells would affect fungal reproduction as current arbuscules senesce 10–12 days after reaching maturity (Toth and Miller 1984).

Our results are consistent with Ca-deficiency symptoms. Both sweet potato and onion showed premature senescence of roots when 11.7 mM $MgSO_4$ was applied. Colonization and sporulation responded in nearly identical ways. The length of time required to develop distinct separation of effects between the highest level of applied $MgSO_4$ and the two other treatments was more consistent with a lack of root cells for colonization than with an immediate physiological shut down and disruption of the symbiosis. It is possible that the Ca-deficiency effects were not apparent earlier because of the transplant shock evident in declining colonized root length and percentage colonization until 4 weeks after transplanting. The findings of these studies support those of Hepper and O'Shea (1984) where lettuce given low Ca was also poorly colonized.

The hydrogen ion concentration in solution could be a significant factor in the interactions described in our work, especially if toxic elements were present in excess and their activity was largely pH dependent (Habte and Soedarjo 1995). However, no dramatic pH changes were recorded during routine maintenance of either aeroponic or sand cultures, even with the large alterations in $MgSO_4$ concentration.

Several differing experimental conditions may explain why our results differ from those of Gryndler et al. (1992). The maximal and minimal temperatures were at least 8 °C higher, which alone should increase the metabolic rate of the mycorrhizas. Likewise, light conditions were generally two- to fourfold more intense. In addition to using different hosts, the fungus we used has been shown to be a good growth promoter (Sylvia et al. 1993) over a broad range of conditions. Tissue-P concentration at the time of response to Mg and Ca was at a level considered deficient for onion growth and conducive for AM colonization (Sylvia and Neal 1990), while the tissue-P concentration in the maize used by Gryndler et al. (1992) was not at a deficient level. Nonetheless, our results had some similarity to Gryndler et al. (1992) in showing the inverse relationship between Mg and Ca levels in tissue; it is possible that the same negative effect on colonization and sporulation would develope in maize with time.

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