

Root Colonization by Arbuscular Mycorrhizal Fungi Does Not Improve Greenhouse Bell Pepper Performance under Water Deficit and Different Nitrogen Regimes

Brooke Matusko¹, Ricardo Hernández¹, Kevin Garcia²,
Rekha Pandey², Amanda A. Cardoso²,
Fernando Fuentes-Peñailillo³, and Emmanuel Torres-Quezada¹

KEYWORDS. biostimulant, controlled environment, physiological stress responses, root colonization

ABSTRACT. Bell pepper (*Capsicum annuum* L.) is a high-value vegetable crop, and its productivity in greenhouse and controlled-environment systems depends strongly on irrigation and nutrient management. Arbuscular mycorrhizal fungi (AMF) have been proposed as biological tools to improve plant water and nutrient acquisition, but their functional value in intensively managed vegetable systems remains uncertain. This study evaluated whether commercial AMF inoculation improves physiological performance, growth, yield, nutrient status, and root colonization in greenhouse-grown bell pepper. Two complementary experiments were conducted using a commercial AMF product containing *Rhizophagus irregularis*, *Funneliformis mosseae*, *Rhizophagus aggregatum*, and *Claroideoglomus etunicatum*. In the first experiment, pot-grown plants were subjected to well-watered or water-deficient irrigation regimes, with or without AMF inoculation at seeding. Water deficiency reduced stomatal conductance, increased leaf temperature, decreased plant height and biomass, and substantially reduced fruit yield relative to well-watered conditions. Although AMF-inoculated plants showed measurable root colonization, inoculation did not improve water consumption, delay wilting, increase stomatal conductance, enhance photochemical efficiency, or mitigate yield losses under water deficit. In the second experiment, the timing and method of AMF application were evaluated at two nitrogen fertilizer rates. Inoculation at seeding and direct placement near the root zone increased AMF colonization compared with later or irrigation-based application methods; however, neither inoculation treatment nor nitrogen rate significantly affected plant height, shoot or root biomass, fruit yield, foliar nutrient status, or time to flowering. Overall, these results indicate that AMF root colonization does not necessarily confer physiological or agronomic benefits in intensively managed greenhouse bell pepper production. The effectiveness of commercial AMF bioinoculants appears to be highly context dependent and may be limited when irrigation and fertilization already provide favorable resource availability.

Introduction

Bell pepper (*Capsicum annuum* L.) is an economically important vegetable crop cultivated in both open-field and protected production systems. While open-field cultivation remains widely used, tunnels, net houses, greenhouses, and controlled-environment systems represent specialized forms of protected production, each with distinct structural, environmental, and management challenges. These high-value systems are particularly important for colored bell peppers and other intensively managed vegetable crops (Flores-Velazquez et al. 2022;

Kabir et al. 2021). While some protective structures buffer crops from extreme climates, they are all still influenced by external weather conditions. Consequently, bell pepper performance in both open-field and protective structures is strongly affected by environmental fluctuations, irrigation regime, and nutrient availability, which influence plant growth, fruit yield, fruit quality, and water and nutrient use efficiency (Byeon et al. 2025). Climate change has further intensified these challenges by increasing the frequency and severity of drought events through elevated peak daytime temperatures and higher crop

evapotranspiration demand (Fuentes-Peñailillo et al. 2025; Milly and Dunne 2016; Naumann et al. 2018; Williams and Abatzoglou 2025). Average annual temperatures and the frequency of events above 100 °F have increased across the United States in recent decades (Diffenbaugh and Ashfaq 2010; Hansen et al. 2012; Portmann et al. 2009). Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms that form symbiotic associations with the roots of most land plants (Parniske 2008). This symbiosis is reported to enhance plants' access to water and nutrients, particularly under conditions of limited or highly variable availability (Balestrini et al. 2018; Bitterlich et al. 2018; Harrison 2012). Mechanistically, AMF extends the effective root absorption zone and induces physiological modifications that improve nutrient acquisition and water uptake (Aroca et al. 2007; Kakouridis et al. 2022; Smith and Smith 2011). In addition, AMF has been reported to support photosynthetic performance in plants by maintaining stomatal function and root hydraulic conductance during periods of water stress (Augé et al. 2015). In bell pepper production, in which yield and fruit quality are highly sensitive to short-term disruptions in water and nutrient supply (Bello et al. 2025), AMF-mediated buffering of plant stress responses may offer a practical strategy to stabilize growth and productivity under greenhouse conditions. Modern greenhouse systems often depend on intensive irrigation and fertilization management in container-based setups (often plastic pots or bags) that constrain root development and use commercially prepared soilless media, fumigated or with microbially reduced conditions (Atzori et al. 2021). The limited volume of growing media in most containers leaves little margin for irrigation errors or system malfunctions before plants experience water stress. Moreover, because bell peppers are commonly propagated in nurseries before field transplanting, the seedling stage offers an operationally suitable window for AMF inoculation. Assessments of AMF bioinoculants in vegetable systems remain limited, and published findings often report inconsistent plant responses to inoculation, with some studies

showing enhanced growth or stress tolerance and others reporting no measurable benefit, even within the same crop species (Kokkoris et al. 2019; Roupahel et al. 2015). In bell pepper, colonization by *Rhizophagus clarus* increased growth and fruit yield under moderate salinity in containerized glasshouse conditions (Kaya et al. 2009). However, these benefits diminished under severe salinity and were minimal under nonstressed conditions, and AMF-inoculated plants exposed to salinity did not perform as well as nonstressed controls (Kaya et al. 2009). Similarly, in cucumber (*Cucumis sativus*), colonization by *Funneliformis mosseae* under chilling stress reduced root colonization compared with optimal temperatures, yet inoculated plants showed greater total fresh mass and increased secondary metabolite accumulation under both optimal and low-temperature conditions (Chen et al. 2013). In tomato (*Solanum lycopersicum*), plant responses were strongly shaped by environmental context and

fungus strain. Under low light and low humidity in glasshouse and hydroponic systems, inoculation with *F. mosseae* did not enhance photosynthetic performance; under high light combined with low atmospheric moisture, mycorrhizal plants experienced greater water stress, reduced electron transport, and lower stomatal conductance, indicating potential carbon costs under unfavorable conditions (Bitterlich et al. 2019). Conversely, another greenhouse study on tomato evaluating multiple *F. mosseae* strains revealed pronounced strain- and cultivar-specific effects, with significant variation in root colonization, growth, yield, and fruit quality (Ullah et al. 2024). Collectively, these studies indicate that AMF application in vegetable systems is not universally beneficial but instead depends on stress intensity, host genotype, and fungus strain selection, highlighting the need for targeted, system-specific research (Guo et al. 2022; Hoeksema et al. 2010). Accordingly, greenhouse experiments are necessary to rigorously assess the performance and reliability of commercial AMF products, particularly for vegetable crops, before broader field-scale adoption (Biel et al. 2021; Faye et al. 2013; Salomon et al. 2022). This study addresses a key knowledge gap by evaluating whether AMF root colonization translates into measurable physiological, nutritional, and agronomic benefits under greenhouse bell pepper production. Two complementary greenhouse trials were conducted to (1) determine the effects of AMF inoculation on plant water use, growth, and yield under well-watered and water-limited conditions; (2) compare application methods and timing of a commercially available AMF product to identify approaches that maximize root colonization; and (3) evaluate AMF influences on bell pepper performance under low and sufficient nitrogen (N) supply. We hypothesized that AMF inoculation would enhance plant growth and yield under water- and nutrient-limited conditions relative to noninoculated controls and that these responses would differ among application methods due to variation in root colonization.

Materials and methods

Plant material

Two greenhouse trials were conducted at the North Carolina State University (NC State) main campus.

Both trials used the same commercial AMF product: MycoApply Ultrafine Endomycorrhizae (Valent BioSciences, Libertyville, IL, USA), which contains a clay carrier mixed with four AMF species: *Rhizophagus irregularis*, *F. mosseae*, *Rhizophagus aggregatum*, and *Claroideoglomus etunicatum*. Bell pepper seeds for both trials were planted, germinated, and maintained under a mist bench at the MaryAnn Fox Greenhouse (Fox Greenhouse) at NC State (lat. 35.7874°N, long. 78.6734°W) for 56 d. The target temperature was set at 24 °C during the day and 18 °C at night. On average, actual greenhouse temperatures did not exceed 29 °C. Relative humidity ranged from 40% to 80%. Bell pepper 'Red Knight' F1 hybrid was seeded into 200-cell black plastic trays (25-cm length × 54-cm width × 4-cm height; cell diameter was 2.3 cm at the top and 1.0 cm at the bottom) containing Sun Gro Metro-Mix 830 soilless substrate (Sun Gro Horticulture, Agawam, MA, USA). The soilless substrate consisted of sphagnum peatmoss, bark, perlite, dolomitic limestone, a wetting agent, and silicon. The substrate was commercially described as having medium drainage, medium particle size, and moderate water-holding capacity. A nutrient analysis of the growing medium was conducted using a fresh sample collected from the substrate before planting. Seedling trays were placed in the greenhouse under an overhead mist bench equipped with Netafim Cool-Net Pro four-nozzle foggers (Netafim Irrigation, Inc., Fresno, CA, USA), spaced 1 m apart and positioned 1 m above the bench surface. Irrigation was managed using a battery-operated Hunter NODE controller (Hunter Industries, San Marcos, CA, USA) connected to a solenoid valve, programmed to mist the trays three times daily for 5 min per event. Seedlings were maintained on the mist bench for 8 weeks and fertilized weekly with Miracle-Gro water soluble-all-purpose plant food (Scotts Miracle-Gro Company, Marysville, OH, USA) at 50 to 100 ppm as they developed.

Experiment 1. Effect of AMF on bell pepper response to contrasting irrigation regimes

The objective of this experiment was to simulate water stress under greenhouse conditions and assess whether

Received for publication 26 Jun 2026. Accepted for publication 12 Aug 2026.

Published online 11 Sep 2026.

¹Department of Horticultural Science, North Carolina State University, Raleigh, NC 27607, USA

²Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695, USA

³Vicerrectoría Académica, Universidad de Talca, Talca 3460000, Chile

We gratefully acknowledge the support of the S.M.A.R.T. initiative and all contributors who assisted during the development of this study. We also thank the technical, greenhouse, and laboratory personnel who supported plant production, experimental setup, irrigation management, sample processing, data collection, and root colonization assessments. We acknowledge North Carolina State University and the Universidad de Talca for providing academic and collaborative support during the preparation of this work.

This research was partially funded by the National Fund for Innovation and Scientific and Technological Development of the Ministry of Higher Education, Science and Technology of the Dominican Republic under Grant 2020-2021-2D5-055 through the project Development of a Low-Cost and Sustainable Production System for Peppers under Protected Environment Conditions in the Dominican Republic.

During the preparation of this article, the authors used AI-assisted language editing tools (Claude Cowork and ChatGPT) solely for language refinement, editorial support, and improvement of text clarity. These tools were not used for study design, data analysis, interpretation of results, or generation of scientific conclusions. We have reviewed and edited the final manuscript and take full responsibility for the content of this publication.

E.T. is the corresponding author. E-mail: eatorre3@ncsu.edu.

This is an open access article distributed under the CC BY-NC license (<https://creativecommons.org/licenses/by-nc/4.0/>).

<https://doi.org/10.21273/HORTTECH05970-26>

AMF inoculation affects plant performance, stress indicators, and plant–water relations. The experiment was conducted using a two-factor factorial arrangement of treatments in a randomized complete block design. The first factor was the irrigation regime, with two levels: well watered and water deficient. The second factor was AMF application, consisting of two levels: AMF applied at seeding and a noninoculated control. Treatments were replicated across six blocks ($n = 6$) to account for spatial variation in temperature and light within the greenhouse. Each potted plant was considered a biological replicate. Well-watered plants were irrigated on a fixed schedule twice daily, with irrigation duration adjusted from 2 to 5 min per event over time to accommodate increasing plant water demand. Water-deficient plants were irrigated only after visible wilting began. Wilting was assessed daily between 1100 and 1400 HR, and plants were watered to container capacity at each irrigation event when necessary. This management approach imposed repeated cycles of water deficit and recovery throughout the experimental period. For the AMF treatments, 0.25 g of the commercial AMF spore product was applied directly to each planting hole at seeding, following the product label instructions. This localized application was selected to maximize physical contact between AMF propagules and the emerging root system, thereby increasing the likelihood of early colonization. Planting holes were manually created to a depth of 0.5 to 1 cm, and the spore product was applied with a precision measuring spoon before seed placement. The seeds were then sown, lightly covered with soilless substrate, and watered in to ensure contact between the inoculum and the seed zone. Noninoculated control treatments did not receive any AMF product. After 8 weeks, the plants were transported from the Fox Greenhouse to a secondary greenhouse at the NC State University Horticulture Field Laboratory (lat. 35.7909°N, long. 78.6977°W), which housed the specialized equipment needed to conduct the remainder of the experiment. Environmental conditions in the second greenhouse were comparable to those in the Fox Greenhouse, although light transmission was lower, ranging from 60% to 80%. No supplemental lighting was used. The seedlings were transplanted into 7.57-L black plastic nursery containers

(24 cm × 23 cm) filled with 2300 g of soilless substrate (Sun Gro Metro-Mix 830), adjusted to 51% moisture content at potting. Six plants per treatment were retained for analysis, resulting in a final analytical sample size of 24 plants. In some cases, irrigation timers failed to activate as scheduled or soil-moisture sensors stopped recording; therefore, only plants with reliable irrigation delivery and continuous weight records were included in the water-use analyses. Following transplanting, the seedlings were maintained under optimal irrigation conditions for 4 weeks to ensure adequate root establishment. During this establishment period, all plants were irrigated to full container capacity and allowed to drain freely. After initiation of irrigation treatments, the plants were grown for an additional 7 weeks until harvest. Throughout the experiment, all plants were fertigated using a piston injector system (Dosatron, D25, 11 gpm; Dosatron, Clearwater, FL, USA) supplying Jack's Professional 20N–10P–20K Peat-Lite (J.R. Peters, Inc., Allentown, PA, USA). The electrical conductivity of the nutrient solution increased from 0.64 to 2.5 dS·m⁻¹ as plant demand increased over time. At 5 weeks after irrigation treatments were initiated, supplemental calcium was applied through the irrigation water at a concentration of 200 ppm to reduce blossom-end rot incidence; consequently, the total amount of calcium received by each plant was determined by its cumulative irrigation volume. To stabilize the plant, a plastic stake was inserted into the growing media ~5 cm from the stem and secured to the plant using Velcro tape. Soil surface evaporation was minimized by covering the media with a polyethylene foam sheet (30.5 cm × 30.5 cm, 0.158-cm thickness) cut to fit the pot diameter. A Decagon 10HS soil moisture sensor (Meter Group, Pullman, WA, USA) was installed in each pot to monitor volumetric water content. Irrigation was delivered using two Netafim (Netafim USA, Fresno, CA, USA) stake drip emitters (2.3 L·h⁻¹ capacity each) per container, positioned on opposite sides of the plant and connected via spaghetti tubing.

LOAD CELL SYSTEM AND WATER CATCHMENT DESIGN. The greenhouse was equipped with a Titan environmental control system (Argus Control Systems, Langley, Canada) with load cell Modbus interface modules to

integrate load cells into the control network (Fig. 1). Container weight for each plant was recorded at 1-min intervals and stored via a computer interface (Advanced Control Solutions, Marietta, GA, USA). The greenhouse has six Titan systems, each controlling three benches, for a total of 18 benches. Each bench was equipped with eight load cells, with four plants installed per bench. The load cells used were Ohaus CKW3R single-platform models with a measurement accuracy of 0.001 lb. To quantify the amount of water used by each plant, a gravity-based water-catchment system was constructed to measure pot mass and leachate runoff separately. Each potted plant was placed on a raised load cell, while drainage was collected on an adjacent unraised load cell. The load cell was mounted on a platform, and two wooden wedges (20 cm × 3.175 cm × 0.635 cm) were placed on the scale to create a slight downslope. A 25-cm × 51-cm black plastic propagation tray, modified with drainage holes drilled into the downslope side, was positioned on the wedges. The plant pot was placed inside the tray on the upslope end to ensure that all leachate flowed toward the drainage holes. The propagation tray was carefully aligned so that its drainage holes emptied directly above a second, unraised load cell. A 25-cm × 25-cm black plastic tray without drainage holes was placed on a second load cell, parallel to the propagation tray, to collect and weigh all leachate runoff from the plant. This design was used to separate plant-container weight changes from drainage losses, thereby improving the estimation of daytime water consumption under contrasting irrigation regimes.

PLANT GROWTH, PHYSIOLOGICAL MEASUREMENTS, AND YIELD ASSESSMENT. Plant height was measured weekly from the official start of the experiment (initiation of irrigation treatments) until termination (7 weeks in total) using a handheld yardstick from the substrate surface to the highest point of the canopy. Stomatal conductance to water (gsw), leaf temperature, and maximum quantum yield of photosystem II (leaf Fv/Fm) were measured during four sampling periods using a handheld porometer/fluorometer (LI-600; LI-COR Biosciences, Lincoln, NE, USA). At each sampling event, visibly wilted plants were



Fig. 1. Greenhouse bell pepper plants grown in individual containers on an automated gravimetric irrigation platform equipped with load cells, irrigation tubing, and drainage collection trays for continuous monitoring of plant water use and controlled irrigation delivery.

counted separately in the two drought treatments, with and without AMF inoculation. The smaller of these two counts was used as the common sample size (n) for that event. Accordingly, n wilted plants were sampled from each drought treatment, and n plants were randomly selected from each well-watered treatment. This approach maintained equal replication across all treatments in the factorial design. Because the number of wilted plants changed over time, the sample size also varied among sampling events. Leaf F_v/F_m was assessed on leaves adapted to darkness for at least 30 min. For leaf water potential determination, fully expanded leaves were collected by cutting the petiole with a razor blade. The samples were immediately placed in sealed plastic bags containing damp paper towels, stored in a cooler, and transported to the

laboratory for analysis. The PMS instrument model 1505D pressure chamber (PMS Instrument Company, Albany, OR, USA) was used to measure leaf water potential by applying controlled gas pressure to excised leaves. Once fruit coloration had initiated across treatments, the entire crop was harvested, with all fruit from each plant collected. Fruits were graded as marketable or unmarketable based on size, shape, and visual quality according to US Department of Agriculture (USDA) bell pepper grading standards (Agricultural Marketing Service—US Department of Agriculture 2025). Fruit number and total fruit weight per plant were recorded. Following harvest, the plants were ended and separated into shoot and root components for biomass determination. Shoot biomass was collected by cutting the stem at the substrate surface,

and the samples were dried in a forced-air oven at 75 °C for 1 to 2 weeks, after which dry weights were recorded. For root biomass determination, the entire root ball was removed from the container, and excess substrate was shaken off. The roots were washed with tap water over a 2.5-mm mesh sieve to minimize biomass loss. Clean roots were air-dried at room temperature for 1 week, sealed in labeled plastic bags, and weighed before AMF colonization analysis.

AMF ROOT COLONIZATION ASSESSMENT. Root colonization by AMF was quantified using a standard staining and microscopy procedure. Segments of ~2 cm of first- and second-order lateral roots were excised from the dried root systems and rehydrated in deionized water for 12 h. Due to excessive root pigment, the samples were cleared in a 10% (w/v) KOH solution for 12 to 14 h at room temperature. Afterward, the used KOH solution was replaced with fresh solution, and the sample was cleared a second time by heating it in a gently boiling water bath at 100 °C for 15 min, followed by three rinses with deionized water. The roots were then stained with Pelikan black ink and an acetic acid (vinegar) solution at 100 °C for 7 min. After staining, the roots were rinsed twice with deionized water and submerged overnight in deionized water to reduce excess stain. Prepared root segments were mounted in water on petri dishes and examined under a dissecting microscope at 10× to 40× magnification. Root colonization was assessed based on the presence or absence of visually identifiable AMF structures within individual root segments. Specific AMF structures, including arbuscules and vesicles, were not independently quantified; therefore, this assessment provides a measure of root colonization but does not directly quantify the functional activity of the mycorrhizal association.

STATISTICAL ANALYSIS. Daytime plant water consumption was calculated from minute-by-minute container weight data obtained via load cells and analyzed in R version 4.5.1 (R Core Team, Vienna, Austria) with the tidyverse and lubridate packages. Individual irrigation events were identified by detecting increases in container weight of 30 g or more between

consecutive 1-min measurements, which were classified as irrigation “peaks.” For well-watered treatments, the plants were irrigated twice daily at 0900 and 1600 HR. Water-use events were defined as beginning 1 h after the morning irrigation peak, at 1000 HR, to allow drainage and postirrigation weight stabilization to occur, and ending at 1530 HR, 30 min before the next scheduled irrigation. This resulted in a standardized 5.5-h water-use period. Because water-deficit treatments were not irrigated on a fixed schedule, their water-use events were defined as beginning 1 h after each detected irrigation peak and continuing for the same 5.5-h duration. Using an equivalent 5.5-h postirrigation interval for both irrigation treatments allowed water use to be compared over a consistent measurement period. Total water consumption per event was calculated as the difference in container weight over the event duration. Hourly water consumption rates ($\text{g}\cdot\text{h}^{-1}$) were calculated by dividing total water use by event duration. Additional statistical analyses were conducted using JMP student edition, version 18.2.2 (SAS Institute Inc., Cary, NC, USA). Repeated measure data, including plant height, stomatal conductance, chlorophyll fluorescence, leaf temperature, leaf water potential, and container weight, were analyzed using generalized linear mixed models (GLMMs) with a normal distribution. Model assumptions were evaluated using residual diagnostic plots, including normal Q-Q plots and plots of studentized residuals against predicted values, to assess normality and homogeneity of variance. The experiment included six blocks, which were incorporated into all models as a random effect. For repeated-measures variables, plant nested within block was included as the experimental subject to account for repeated observations on the same plant over time. Fixed effects included irrigation regimen, AMF application, sampling order, and their interactions. Single-measurement response variables, including aboveground biomass, root biomass, fruit yield, days between wilting events, and cumulative water consumption, were analyzed using mixed-effects models with a normal distribution and fixed effects of row, irrigation regimen, AMF application, and their interaction. The percentage

Table 1. Treatments to evaluate the effect of the application method of arbuscular mycorrhizal fungi on bell pepper under different nitrogen supply levels.

Treatment	Application method and timing		Nitrogen rate ($\text{kg}\cdot\text{ha}^{-1}$) ⁱ
	Seeding	Transplant	
1	NA (control)	NA (control)	134.5
2	Planting hole	NA	134.5
3	Irrigation water	NA	134.5
4	Mix in media	NA	134.5
5	NA	Planting hole	134.5
6	NA	Irrigation	134.5
7	NA (control)	NA (control)	67.25
8	Planting hole	NA	67.25
9	Irrigation water	NA	67.25
10	Mix in media	NA	67.25
11	NA	Planting hole	67.25
12	NA	Irrigation	67.25

ⁱNitrogen rates were adjusted using different concentrations of a 20N-10P-20K all-purpose water-soluble fertilizer.

NA = not applicable.

of AMF root colonization was analyzed using a GLMM with a binomial distribution to estimate the probability of colonization. All models generated descriptive statistics, including means, standard deviations, and 95% confidence intervals. When significant main effects or interactions were detected, treatment means were separated using Fisher's least significant difference test at $\alpha = 0.05$.

Experiment 2. Effects of AMF inoculation and N rate on bell pepper growth and performance

The objective of this experiment was to evaluate whether the method and timing of AMF application influenced root colonization and overall plant performance under contrasting N fertility regimes. Bell pepper plants were inoculated with AMF spores either at seeding or at transplanting and grown through harvest to assess treatment effects. Plant performance indicators were measured at key developmental stages, including vegetative growth, flowering, and fruiting. The experiment employed a split-plot design with three factors. The whole-plot factor was N fertilizer rate, with two levels: 67.25 and 134.5 $\text{kg}\cdot\text{ha}^{-1}$. The subplot factors consisted of AMF application method with four levels (noninoculated control, planting hole, growing media, and irrigation water) and application timing with two levels (at seeding or at transplanting). This resulted in six AMF treatment combinations evaluated at each N level, for a total of 12 treatment combinations,

including one control treatment per N rate (Table 1). Blocking was used to account for spatial variation in temperature and light within the greenhouse.

The AMF spore product described above was applied using three inoculation methods: (1) direct placement in the planting hole, (2) application through irrigation water, and (3) incorporation into the growing media. For the planting-hole treatment at seeding, 0.25 g of AMF product was placed directly into each planting hole using a measuring spoon. The holes were manually created to a depth of 0.5 to 1.0 cm, after which seeds were placed and lightly covered with soilless substrate. New transplants, grown as described above, were used, and 1.0 g of the AMF product was applied to each planting hole at transplanting. The holes were dug to a depth of ~4 cm, corresponding to the size of the transplant plug, and the inoculum was placed in the hole immediately before transplanting.

For the irrigation-water treatment, an AMF suspension was prepared at a concentration of 12 g of product per gallon (3.78 L) of water. Following seeding, germination trays were irrigated with the suspension using overhead irrigation until container capacity was reached. For treatments receiving AMF at transplanting, the same suspension was applied immediately after transplanting by irrigating each container, up to container capacity.

For the growing-media treatment, the AMF product was thoroughly incorporated into the soilless substrate at

a rate of $8.5 \text{ g}\cdot\text{ft}^{-3}$ ($300 \text{ g}\cdot\text{m}^{-3}$) before filling germination trays. The substrate was mixed until a homogeneous distribution of spores was achieved and then used for seeding. This application method was used only during seedling production and was not evaluated at transplanting because incorporation into container media is generally impractical under commercial production conditions.

The seedlings were transplanted at the Fox Greenhouse into 7.57-L black nursery containers (24 cm $\times$ 23 cm) filled with Sun Gro Metro-Mix 830 soil-less substrate at $\sim 50\%$ moisture content at the time of planting. AMF treatments applied at transplant included planting-hole application and irrigation-water application. A total of 40 seedlings were transplanted per treatment, resulting in 480 plants in the experiment. Each container was equipped with a single Netafim stake drip emitter positioned near the root zone. Irrigation was scheduled using a battery-operated Hunter NODE controller and applied four times daily at 0700, 1000, 1300, and 1600 HR. Irrigation duration ranged from 2 to 5 min throughout the growing period as plant water demand increased and was adjusted to prevent leaching through the container's drainage holes. The plants were horizontally trellised using tomato twine supported by metal rods attached to the greenhouse benches. The plants were fertigated weekly at N rates equivalent to either $67.25 \text{ kg}\cdot\text{ha}^{-1}$ (50% of the average recommended rate for bell pepper) or $134.5 \text{ kg}\cdot\text{ha}^{-1}$ (100% of the average recommended rate for bell pepper without mulching, according to a southeast US vegetable production handbook) (Kemble 2025). Fertilizer solutions were prepared by weighing and dissolving 20N-10P-20K all-purpose soluble fertilizer in water to achieve the target N concentration. Each plant received 100 mL of the concentrated fertilizer solution once per week during morning hours. From transplanting, the plants were grown for 13 weeks until harvest initiation.

PLANT GROWTH, YIELD, AND BIOMASS MEASUREMENTS. Plant height was measured every 2 weeks using a handheld yardstick from the substrate surface to the highest point of the canopy. Aboveground biomass samples were collected biweekly and at final harvest by cutting the stems at the

substrate surface and placing the samples in paper bags. The samples were dried in a forced-air oven at 75°C for 1 to 2 weeks and then weighed. Leaf tissue samples collected at termination were dried using the same protocol and subsequently processed for foliar nutrient analysis. Days to flowering after transplanting were recorded as the time point at which 50% of the plants in a treatment exhibited fully open flowers. Fruit harvest occurred on three occasions at 1-week intervals. Only fruit that had initiated color change or had fully colored were harvested. Fruits were graded as marketable or unmarketable based on size, shape, and visual appearance according to USDA bell pepper grading standards. Both the number of fruit and the fresh weight per plant were recorded. Following the final harvest, all plants were ended and separated into shoot and root components for further analysis, as previously

described. Root biomass was determined by carefully removing the entire root ball from the container and shaking off excess substrate. The roots were washed with tap water over a 2.5-mm mesh sieve to minimize biomass loss. Clean roots were air-dried at room temperature for 1 week, sealed in labeled plastic bags, and weighed before AMF root colonization analysis.

AMF ROOT COLONIZATION QUANTIFICATION. AMF root colonization was assessed using the same clearing and staining procedure as outlined for experiment 1.

STATISTICAL ANALYSIS. Statistical analyses were conducted using JMP student edition, version 18.2.2 (SAS Institute Inc.). The Fit Model platform was used to evaluate data distributions and explore relationships among experimental factors, with results dynamically linked to statistical summaries. Plant height and fruit yield were analyzed as repeated-measures responses

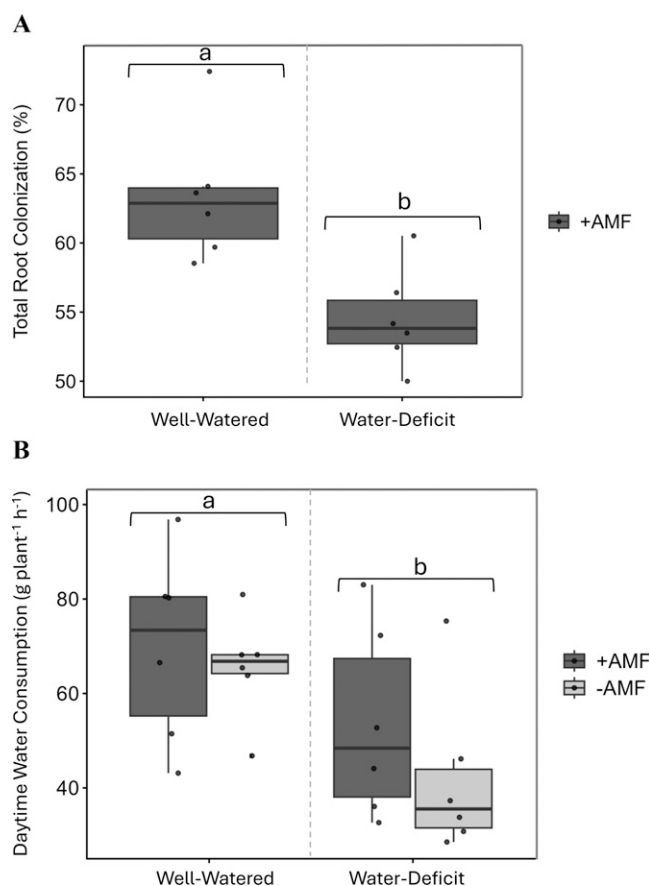


Fig. 2. (A) Percentage of root colonization of bell pepper plants by arbuscular mycorrhizal fungi (AMF) under well-watered and water-deficient irrigation ($P \leq 0.0320$). (B) Effect of irrigation and AMF inoculation on bell pepper daytime water consumption under greenhouse conditions ($P \leq 0.0225$). The data include six biological replicates, and different letters indicate treatments with significantly different means (Fisher's least significant difference $\alpha = 0.05$).

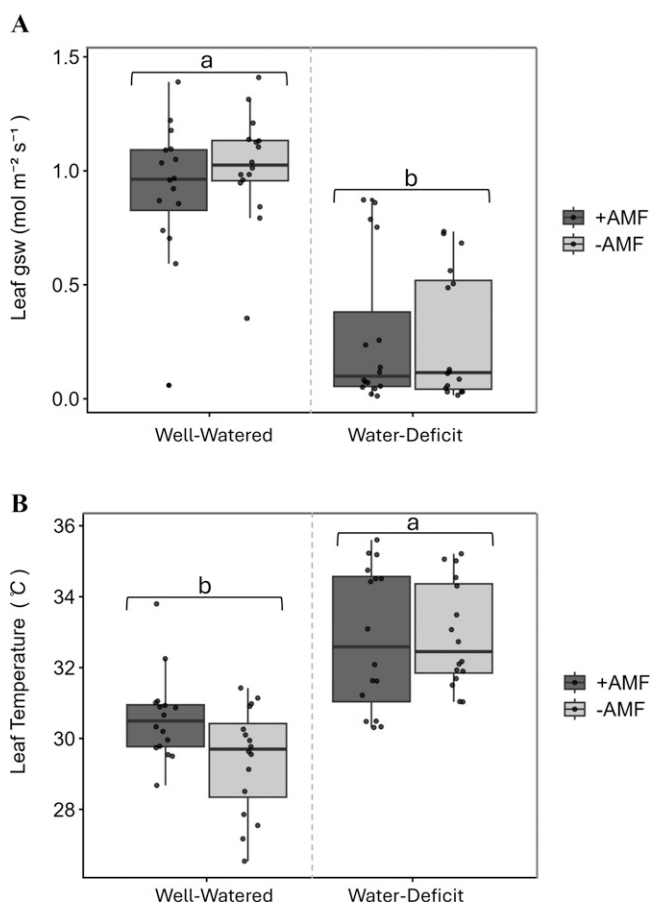


Fig. 3. Effect of irrigation levels and arbuscular mycorrhizal fungi (AMF) inoculation on stomatal conductance to water (leaf gsw) ($P \leq 0.0001$) (A) and leaf temperature in bell pepper plants under greenhouse conditions ($P \leq 0.0001$) (B). The data include four biological replicates per treatment, evaluated at four sampling times ($n = 16$). For water-limited treatments, plants with visible wilting were selected for measurements. Different letters indicate treatments with significantly different means (Fisher's least significant difference $\alpha = 0.05$).

using generalized linear mixed models with a normal distribution. Shoot biomass collected across sampling dates was analyzed with sampling time as a fixed effect. Final root biomass and foliar nutrient concentrations were analyzed as single-measurement variables using mixed-effects models with normal distributions. Fixed effects included AMF inoculation treatment, fertilizer rate, weeks after transplanting, and all corresponding two- and three-way interactions. Block and the appropriate whole-plot and subplot error terms were incorporated into the models to account for the split-plot experimental design. Percent AMF root colonization was analyzed using a GLMM with a binomial distribution. For this analysis, each root segment was classified as colonized or not colonized based on the visual presence of AMF structures, and the model estimated

the probability of colonization. Days to flowering were analyzed using a GLMM with an exponential distribution because the response represented time until an event occurred. Flowering was considered to have occurred when at least 50% of the plants within a treatment reached the flowering stage. For all response variables, descriptive statistics, including means, standard deviations, and 95% confidence intervals, were calculated. When significant treatment effects were detected, the means were separated using Fisher's least significant difference test at $\alpha = 0.05$.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Results

Experiment 1

AMF did not mitigate the negative impact of limited irrigation on plant function, growth, and production. Fungal structures associated with AMF colonization were not detected in noninoculated plants, indicating that the selected growing medium did not contain detectable native AMF under the staining and microscopy conditions used in this study. In contrast, AMF-inoculated plants exhibited measurable root colonization, and colonization levels differed significantly between irrigation treatments (Fig. 2). Well-watered plants showed ~9% greater root colonization than water-limited plants, indicating that the irrigation regime influenced AMF establishment and persistence within the root system. Despite differences in AMF colonization, inoculation did not significantly alter the frequency of visible wilting events under water-deficit conditions. Among water-limited plants, the average number of days to wilting was similar between the inoculated and noninoculated treatments, with mean intervals of 4.33 and 4.15 d, respectively. These results suggest that under the imposed cyclic drought stress, AMF inoculation did not delay the onset of visible wilting symptoms. Daytime water consumption rates were quantified for each treatment using load-cell measurements collected during the morning and late-afternoon irrigation periods. AMF inoculation did not significantly affect water consumption rates within either irrigation regime. In contrast, irrigation treatment had a pronounced effect on water use, with well-watered plants exhibiting a 42% higher rate of water consumption (g/plant/h) compared with water-limited plants (Fig. 2). This difference reflects the imposed irrigation management strategy and confirms the effectiveness of the water-deficit treatment in reducing plant water use.

The gsw was not significantly affected by AMF inoculation. Across irrigation treatments, AMF-inoculated and noninoculated plants exhibited mean gsw values of 0.567 and 0.646 mol m⁻² s⁻¹, respectively. In contrast, the irrigation regime had a strong effect on gsw, with well-watered plants exhibiting 312% higher gsw than water-limited plants (Fig. 3). Leaf temperature was similarly

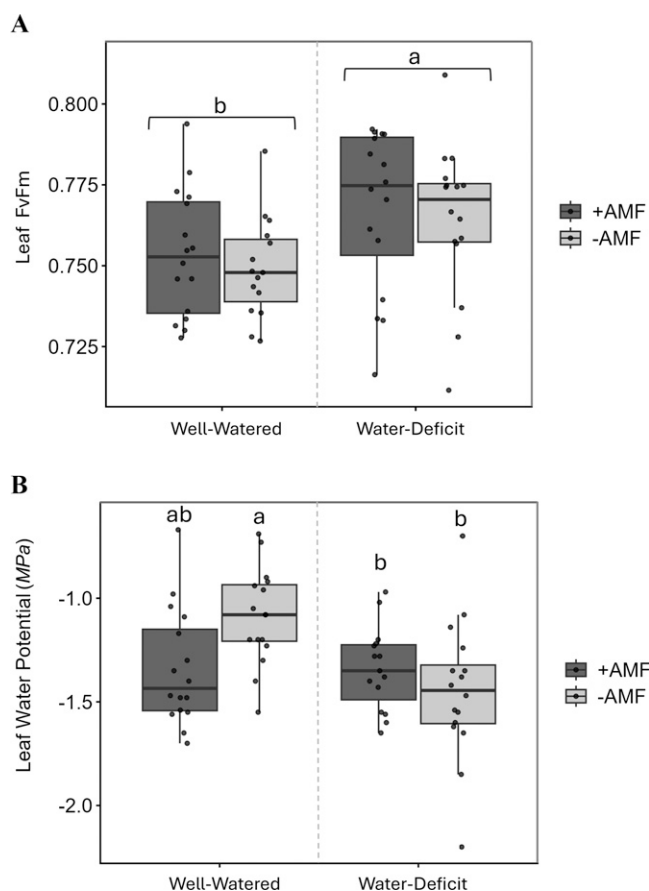


Fig. 4. Effect of irrigation levels and arbuscular mycorrhizal fungi (AMF) inoculation on maximum quantum yield of photosystem II (Leaf Fv/Fm) ($P \leq 0.0017$) (A) and leaf water potential in bell pepper plants under greenhouse conditions ($P \leq 0.0020$) (B). The data include four biological replicates per treatment, evaluated at four sampling times ($n = 16$). For water-limited treatments, plants with visible wilting were selected for measurements. Different letters indicate treatments with significantly different means (Fisher's least significant difference $\alpha = 0.05$).

unaffected by AMF inoculation, with mean values of 31.6 and 31.5 °C observed for inoculated and noninoculated treatments, respectively. However, the irrigation regime significantly influenced leaf temperature, as leaves of well-watered plants were ~10% cooler than those of water-limited plants (Fig. 3). These differences reflect the contrasting plant water status imposed by the irrigation treatments.

In this study, leaf Fv/Fm was not significantly affected by AMF inoculation, with mean values of 0.7625 and 0.7572 observed for inoculated and non-inoculated plants, respectively (Fig. 4). These values indicate no severe photochemical impairment across treatments and no detectable effect of AMF on photosystem II efficiency under the imposed conditions. Water-limited plants continued to exhibit high leaf Fv/Fm values (Fig. 4), indicating that although the

water-deficit regime affected gsw and leaf temperature, it was not severe enough to cause severe PSII impairment. Differences in leaf Fv/Fm between well-watered and water-limited plants were minor and should not be interpreted as evidence that water limitation improved plant health. A significant interaction between AMF inoculation and irrigation regime was observed for leaf water potential (Fig. 4). Under water-deficit conditions, leaf water potential of AMF-inoculated plants was statistically similar to that of well-watered plants receiving AMF, whereas noninoculated water-deficit plants exhibited lower values than those of well-watered plants. Across treatments, water-deficient plants displayed leaf water potential values that differed by ~15% from those of well-watered plants. Similarly, plants inoculated with AMF under well-watered conditions showed

a slight tendency toward a higher negative pressure than plants without AMF. This interaction indicates that the irrigation regime strongly influenced plant water status and that AMF inoculation was associated with a modified leaf water potential response under water-deficit conditions. However, this physiological response did not coincide with improvements in plant growth, water consumption, or fruit yield.

Bell pepper canopy height was not significantly affected by AMF inoculation under either irrigation regime. When averaged across irrigation treatments, AMF-inoculated plants reached a mean height of 43.03 cm, whereas noninoculated plants averaged 42.4 cm. In contrast, the irrigation regime significantly influenced canopy height, with well-watered plants exhibiting a 21% greater height compared with plants subjected to water-deficit conditions (Fig. 5). Similarly, average fruit weight per plant was not significantly affected by AMF inoculation across irrigation treatments. AMF-inoculated plants produced an average of 582.3 g of fruit per plant, while noninoculated plants averaged 513.6 g. The irrigation regime had a pronounced effect on fruit yield, with well-watered plants producing ~50% greater fruit mass per plant than water-deficient treatments (Fig. 5). Aboveground biomass was not significantly influenced by AMF inoculation under either irrigation regime. When averaged across irrigation treatments, AMF-inoculated plants produced a mean aboveground biomass of 54.67 g, while noninoculated plants averaged 52.7 g. In contrast, the irrigation regime had a significant effect on aboveground biomass, with well-watered plants exhibiting 61% greater biomass compared with plants grown under water-deficit conditions (Fig. 6). Root biomass followed a similar pattern, with the irrigation regime emerging as the only factor significantly affecting growth (Fig. 6). Plants maintained under well-watered conditions produced root biomass that was ~22% greater than that of water-deficit plants. No significant effect of AMF inoculation on root biomass was detected within either irrigation treatment.

EXPERIMENT 2. AMF did not improve plant development or yield. Bell pepper root colonization by AMF was

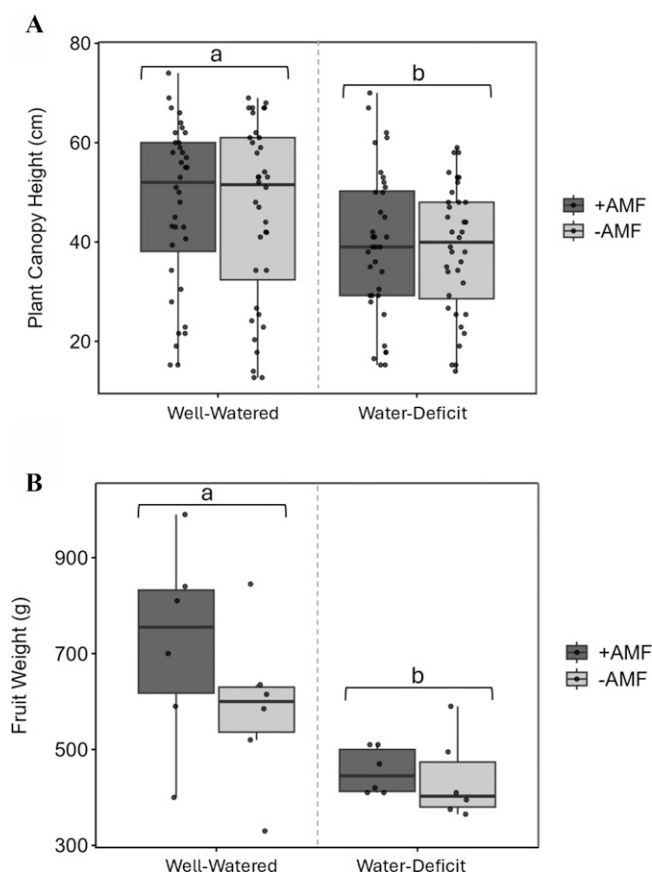


Fig. 5. Effect of irrigation levels and arbuscular mycorrhizal fungi (AMF) inoculation on plant height over the growing period ($P \leq 0.0006$) (A) and fruit weight at harvest of bell pepper plants under greenhouse conditions ($P \leq 0.0027$) (B). The data include six biological replicates, and different letters indicate treatments with significantly different means (Fisher's least significant difference $\alpha = 0.05$).

significantly influenced by N fertilizer rate, with plants receiving $67.25 \text{ kg} \cdot \text{ha}^{-1}$ of N exhibiting ~ 8 percentage points greater root colonization compared with those receiving $134.5 \text{ kg} \cdot \text{ha}^{-1}$ (Fig. 7). Inoculation method also had a significant effect on colonization. AMF application at seeding resulted in higher colonization rates than at transplant, with mean colonization values of 57% and 49%, respectively, corresponding to an increase of ~ 8 percentage points when inoculation occurred at seeding. Differences between AMF application methods were also observed. Both the planting-hole application at seeding and incorporation of AMF into the growing media resulted in significantly greater root colonization compared with application via irrigation at transplant (Fig. 7). These results indicate that both N fertility level and the timing and method of AMF application substantially influenced the extent of root colonization in greenhouse-grown bell pepper. Bell pepper foliar

nutrient concentrations also differed significantly between N fertilizer treatments for all measured elements except phosphorus and manganese (Table 2). Foliar nutrient concentrations differed between N fertilizer treatments, but several nutrients were outside the established sufficiency ranges for bell pepper. Plants receiving $67.25 \text{ kg} \cdot \text{ha}^{-1}$ of N exhibited elevated S, B, and Zn concentrations, whereas plants fertilized at $134.5 \text{ kg} \cdot \text{ha}^{-1}$ of N showed elevated N, K, Mg, Ca, S, B, and Zn concentrations, along with Cu concentrations below the sufficiency threshold (Table 2). No consistent effects of AMF inoculation method on foliar nutrient concentrations were detected. Time to flowering following transplanting was not significantly influenced by N fertilizer rate, AMF inoculation method, or their interaction (Table 3). Across all treatments, plants reached flowering at an average of 37.6 d after transplant, indicating similar developmental

timing regardless of fertility level or inoculation strategy.

Fruit yield was not significantly influenced by N fertilizer rate, AMF inoculation method, or their interaction (Table 3). The absence of yield differences among N rates suggests that N availability was sufficient even at the lowest rate evaluated, with additional N providing no measurable yield benefit. Because the N rates used in this study were based on open-field recommendations, these results suggest that directly transferring field-based N rates to greenhouse production may overestimate crop N requirements. Greater control of irrigation and nutrient delivery under greenhouse conditions can reduce nutrient losses and improve N-use efficiency, potentially allowing comparable productivity with lower N inputs. Likewise, although AMF colonized the roots, the symbiosis provided little additional agronomic benefit when nutrient availability was already sufficient. The absence of treatment effects on canopy height and shoot and root biomass further supports this interpretation. Mean fruit weight declined progressively across harvests, from 151 g in the first harvest to 121 g in the second and 94.16 g in the third. Because this decline occurred across treatments, plant developmental stage or changes in environmental conditions likely exerted a greater influence on fruit growth later in the production cycle than N supply or AMF inoculation. This interpretation is also consistent with the reduction in canopy height growth after week 7, which coincided with fruit development and harvest and likely reflected a shift in assimilate allocation from vegetative to reproductive growth.

Discussion

Across two complementary greenhouse experiments, AMF inoculation resulted in measurable root colonization but did not consistently improve bell pepper growth, physiological performance, yield, nutrient status, or phenological development under either contrasting irrigation regimes or N fertility levels. Neither the timing nor the method of commercial AMF application altered plant height, biomass accumulation, fruit yield, or time to flowering, despite clear treatment effects on AMF establishment. Collectively, these results indicate that,

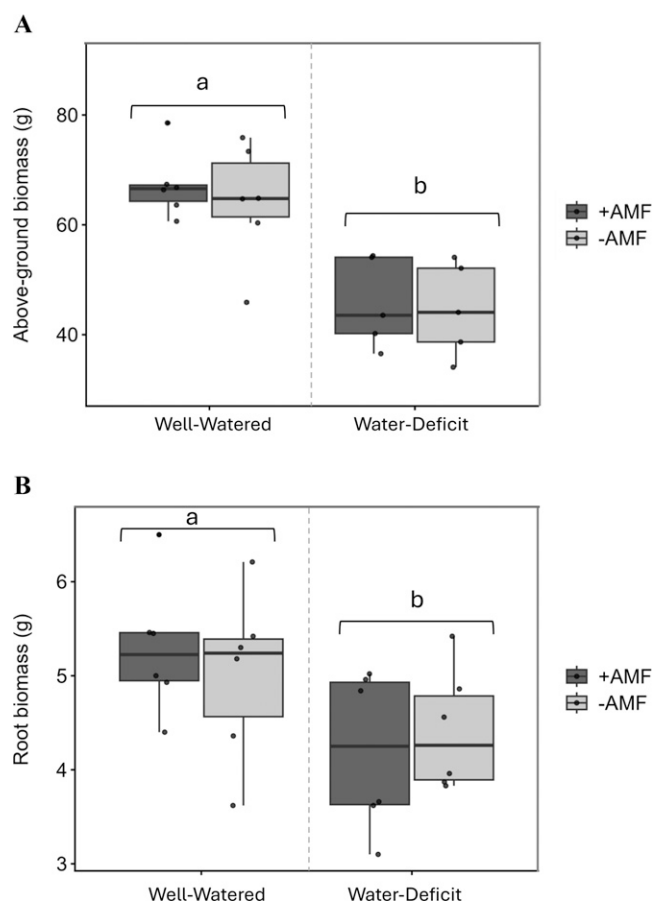


Fig. 6. Effect of irrigation levels and arbuscular mycorrhizal fungi (AMF) inoculation on dry aboveground biomass ($P \leq 0.0001$) (A) and dry root biomass of bell pepper plants under greenhouse conditions ($P \leq 0.0161$) (B). The data include six biological replicates, and different letters indicate treatments with significantly different means (Fisher's least significant difference $\alpha = 0.05$).

under intensively managed greenhouse conditions, AMF colonization alone is insufficient to confer agronomic benefits to bell pepper. Nitrogen fertilizer

rate also did not significantly affect plant growth or yield, as similar performance was observed at 67.25 and 134.5 kg·ha⁻¹. This outcome likely

reflects the high efficiency of irrigation and fertigation management in greenhouse systems, where nutrients are supplied frequently and losses are minimized, resulting in consistently adequate nutrient availability across treatments (Carrasco et al. 2024). Despite the lack of plant performance responses, AMF root colonization was strongly influenced by application method and timing. Inoculation at seeding consistently resulted in greater colonization than inoculation at transplant, while planting-hole application and incorporation into the growing media generally resulted in greater colonization than irrigation-water application. These patterns indicate that early physical proximity between AMF propagules and developing roots favored colonization in this containerized system (Grünfeld et al. 2022). However, the colonization assessment used in this study quantified the presence of AMF structures within roots but did not quantify arbuscule abundance or other indicators of functional nutrient exchange (Luginbuehl and Oldroyd 2017; Roth and Paszkowski 2017; Wang et al. 2017). Therefore, although the commercial inoculant produced detectable root colonization, the functional status of the resulting association cannot be determined from colonization percentage alone. This represents an important limitation when interpreting the absence of physiological or agronomic responses.

High nutrient availability and repeated stress cycles may suppress

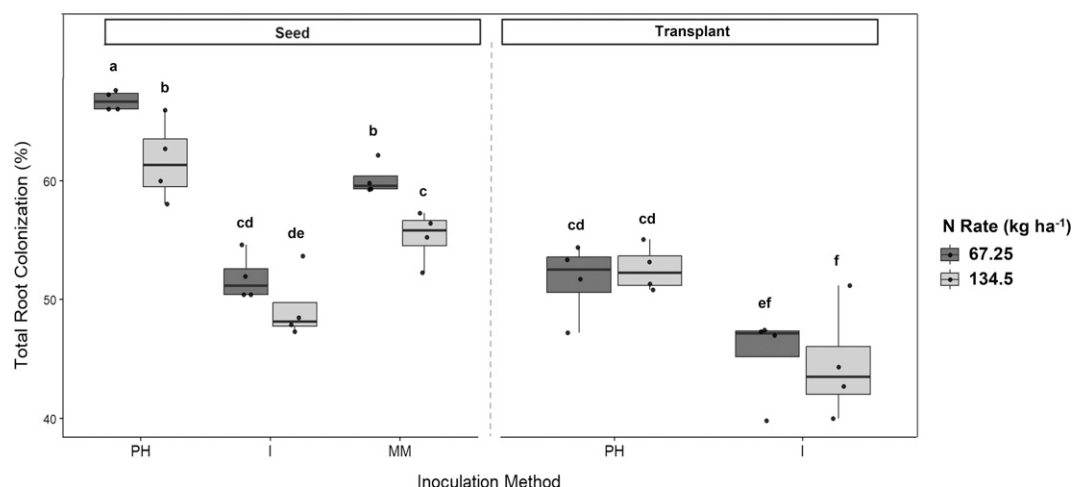


Fig. 7. Effect of nitrogen fertilizer rates (kg·ha⁻¹) and arbuscular mycorrhizal fungi (AMF) inoculation method on root colonization rates in bell pepper plants grown under greenhouse conditions. Inoculation methods include AMF applied to seed at the time of seeding (seed) and AMF applied to transplant at the time of transplanting (transplant). Application was to the planting hole (PH), via irrigation (IR), or directly into the media mix (MM). The data include six biological replicates, and different letters indicate treatments with significantly different means (Fisher's least significant difference $\alpha = 0.05$).

Table 2. Effect of two nitrogen rates on bell pepper foliar nutrient content under greenhouse conditions, in Raleigh, NC, in 2025.

Nutrient and <i>P</i> value	Nitrogen rates and recommended range		
	67.25 kg·ha ⁻¹	134.5 kg·ha ⁻¹	Range
N % (<i>P</i> ≤ 0.0001)	4.43 b	5.37 a	3% to 5%
P % (<i>P</i> ≤ 0.1496)	0.37	0.34	0.3% to 0.6%
K % (<i>P</i> ≤ 0.0003)	4.47 b	5.38 a	3% to 5%
Mg % (<i>P</i> ≤ 0.0005)	0.71 b	1.02 a	0.3% to 0.6%
Ca % (<i>P</i> ≤ 0.0109)	2.38 b	2.72 a	1.5% to 2.5%
S % (<i>P</i> ≤ 0.0001)	0.95 a	0.75 b	0.3% to 0.4%
B ppm (<i>P</i> ≤ 0.0003)	80.17 a	66.83 b	20 to 60 ppm
Zn ppm (<i>P</i> ≤ 0.0009)	125.01 a	100.50 b	25 to 50 ppm
Mn ppm (<i>P</i> ≤ 0.0709)	90.17	104.17	30 to 150 ppm
Fe ppm (<i>P</i> ≤ 0.0201)	125.67 b	155.33 a	50 to 200 ppm
Cu ppm (<i>P</i> ≤ 0.0119)	6.17 a	4.83 b	5 to 20 ppm

Different letters among N rate columns for each nutrient indicate significant differences according to Fisher's least significant difference ($\alpha = 0.05$).

AMF functionality, providing a likely explanation for the disconnection between colonization intensity and plant response. Results from the irrigation experiment further reinforce that water availability was the dominant factor controlling bell pepper physiological responses and productivity. Well-watered plants exhibited greater biomass accumulation, fruit yield, and AMF colonization than water-limited plants, whereas water-deficit treatments were characterized by reduced gsw, elevated leaf temperature, and substantially lower yields. Although AMF inoculation did not significantly affect gsw, daytime water consumption, or the frequency of visible wilting events, a significant interaction between AMF inoculation and irrigation regime was observed for leaf water potential. Under water-deficit conditions, AMF-inoculated plants

showed leaf water potential values comparable to those of well-watered plants receiving AMF, while noninoculated water-deficit plants exhibited lower values. This suggests a limited physiological effect of AMF on plant water status during stress. However, this response did not translate into improved stomatal conductance, water consumption, growth, or yield, indicating that the observed change in leaf water potential was insufficient to confer an agronomic benefit under the conditions of this study. These findings underscore that even moderate, repeated water stress can significantly reduce bell pepper productivity and that irrigation management remains the primary determinant of yield in greenhouse systems. The absence of AMF-mediated improvements in stomatal conductance and

daytime water use during well-watered and water-limited conditions contrasts with numerous reports on field crops, in which AMF have been shown to enhance drought resistance and water-use efficiency under more severe or prolonged droughts (Duan et al. 2024; Oliveira et al. 2022; Xiao et al. 2023). Differences in crop species, stress intensity, substrate volume, nutrient regime, and carbon availability likely account for these contrasting outcomes. Similarly, results from the N experiment demonstrated that foliar nutrient concentrations were driven by fertilizer rate rather than AMF inoculation. Although AMF are commonly associated with enhanced nutrient uptake, particularly phosphorus, no consistent effects of AMF on foliar nutrient status were observed. High baseline levels of P and K, typical of greenhouse vegetable production, may have reduced plant reliance on mycorrhizal nutrient acquisition pathways. This finding aligns with reports on maize and other crops showing that AMF benefits can be neutral or even negative under high-input conditions, under which nutrient imbalances or carbon costs outweigh potential gains (Thielicke et al. 2023). In intensive vegetable systems, this creates a fundamental tension: nutrient regimes required to maximize yield may inherently limit AMF functionality. The limited effectiveness of AMF observed in this study appears to be closely tied to the characteristics of intensively managed greenhouse vegetable systems. High nutrient availability, frequent and precise irrigation,

Table 3. Effect of nitrogen rates and AMF inoculation method on plant height, dry biomass, fruit yield, and time to flowering in bell pepper plants cultivated under greenhouse conditions, in Raleigh, NC, in 2025.

Conditions	Biomass (g/plant)			Fruit yield (g/plant)	Days to flowering
	Plant height (cm)	Shoot	Root		
Fertilizer rate					
67.25 kg·ha ⁻¹ nitrogen	90.68	14.04	8.08	1004.88	38
134.5 kg·ha ⁻¹ nitrogen	99.92	15.84	7.68	995.97	37
Significance	ns	ns	ns	ns	ns
Inoculation method					
Control	94.87	15.07	7.83	1014.37	37
Seed planting hole	95.22	15.11	8.05	988.75	38
Seed irrigation	96.75	14.89	7.84	1001.77	38
Seed media mix	96.72	14.96	7.77	1040.83	38
Transplant planting hole	95.28	15.21	8.08	1044.75	37
Transplant irrigation	92.96	14.40	7.79	912.08	38
Significance	ns	ns	ns	ns	ns
Interaction					
Fertilizer rate × inoculation method	ns	ns	ns	ns	ns

AMF = arbuscular mycorrhizal fungi; ns = nonsignificant according to Fisher's least significant difference ($\alpha = 0.05$).

soilless substrates, and restricted root volume are likely to reduce plant dependence on mycorrhizal-mediated resource acquisition (Baum et al. 2015; Fuentes-Peñailillo et al. 2024; Gruda 2019; McNew et al. 2025). In addition, the carbon cost of maintaining AMF symbiosis may outweigh potential benefits when water and nutrients are readily available (Kiers et al. 2011). Evidence of this trade-off was suggested by trends in leaf water potential under well-watered conditions, under which AMF-inoculated plants tended to exhibit more negative values than noninoculated plants, suggesting a possible physiological cost in resource-rich environments. Under the specific environmental conditions, fertility regimes, and commercial AMF product evaluated in this study, AMF inoculation did not enhance plant function, nutrient uptake, or yield performance in water-limited greenhouse-grown bell pepper. These findings do not negate the potential value of AMF in vegetable production; rather, they highlight the context-dependent nature of mycorrhizal benefits and the need for system-specific evaluation (Torres-Quezada et al. 2025). In intensively managed greenhouse systems, in which plant stress is minimized through precise control of irrigation, nutrition, temperature, light, and humidity, the functional role of AMF may be inherently constrained.

Conclusions

This study evaluated the performance of a commercial AMF inoculant in greenhouse-grown bell pepper across contrasting irrigation regimes, N fertility levels, and application strategies. Measurable AMF root colonization occurred and varied with application timing, method, and fertility level; however, these differences did not translate into consistent improvements in plant growth, physiological performance, fruit yield, plant nutrition, or developmental timing. Although root colonization alone does not demonstrate that a fully functional mycorrhizal symbiosis was established, the presence of AMF structures within the roots confirms that the inoculant reached and colonized the root system. Under this premise, AMF inoculation did not provide measurable improvements in bell pepper performance under the greenhouse conditions evaluated. Irrigation

regime remained the dominant factor affecting plant water use, growth, and productivity, and AMF inoculation did not mitigate the negative effects of water limitation. Likewise, AMF inoculation did not improve plant nutritional status despite measurable root colonization. Therefore, while the functional status of the mycorrhizal association cannot be determined from colonization measurements alone, the overall agronomic outcome was clear: application of the commercial AMF inoculant did not improve crop performance relative to noninoculated plants under the management conditions tested. Future studies should incorporate direct assessments of arbuscule abundance or other indicators of functional nutrient exchange while evaluating AMF under production systems in which biological mediation of water and nutrient acquisition may play a greater role. Overall, these findings provide a useful benchmark for growers and researchers and support a more targeted, evidence-based approach to AMF integration in greenhouse vegetable production.

References cited

- Agricultural Marketing Service—US Department of Agriculture. 2025. Grades of sweet peppers. <https://www.ams.usda.gov/grades-standards/sweet-peppers-grades-and-standards>. [accessed 10 Jul 2025].
- Aroca R, Porcel R, Ruiz-Lozano JM. 2007. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytol.* 173(4):808–816. <https://doi.org/10.1111/j.1469-8137.2006.01961.x>.
- Atzori G, Pane C, Zaccardelli M, Cacini S, Massa D. 2021. The role of peat-free organic substrates in the sustainable management of soilless cultivations. *Agronomy*. 11(6):1236. <https://doi.org/10.3390/agronomy11061236>.
- Augé RM, Toler HD, Saxton AM. 2015. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: A meta-analysis. *Mycorrhiza*. 25(1):13–24. <https://doi.org/10.1007/s00572-014-0585-4>.
- Balestrini R, Chitarra W, Antoniou C, Ruocco M, Fotopoulos V. 2018. Improvement of plant performance under water deficit with the employment of biological and chemical priming agents. *J Agric Sci.* 156(5):680–688. <https://doi.org/10.1017/S0021859618000126>.
- Baum C, El-Tohamy W, Gruda N. 2015. Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review. *Sci Hortic.* 187:131–141. <https://doi.org/10.1016/j.scienta.2015.03.002>.
- Bello AS, Huda S, Chen ZH, Alsafran M, Abdellatif M, Ahmed T. 2025. Maximizing crop yield and economic benefit through water and nitrogen optimization in bell pepper. *Agric Water Manage.* 312:109447. <https://doi.org/10.1016/j.agwat.2025.109447>.
- Biel C, Camprubí A, Lovato PE, Calvet C. 2021. On-farm reduced irrigation and fertilizer doses, and arbuscular mycorrhizal fungal inoculation improve water productivity in tomato production. 288:1100337. <https://doi.org/10.1016/j.scienta.2021.110337>.
- Bitterlich M, Franken P, Graefe J. 2018. Arbuscular mycorrhiza improves substrate hydraulic conductivity in the plant available moisture range under root growth exclusion. *Front Plant Sci.* 9:339760. <https://doi.org/10.3389/fpls.2018.00301>.
- Bitterlich M, Franken P, Graefe J. 2019. Atmospheric drought and low light impede mycorrhizal effects on leaf photosynthesis—A glasshouse study on tomato under naturally fluctuating environmental conditions. *Mycorrhiza*. 29(1):13–28. <https://doi.org/10.1007/s00572-018-0872-6>.
- Byeon SE, Park H, Latt TT, Jeong S, Lee J, Lwin HP, Lee J. 2025. Seasonal fluctuation of fruit quality is differentially affected by mediating mineral nutrients and targeted major metabolites in greenhouse grown bell pepper (*Capsicum annuum* L.) cultivars. *Sci Hortic.* 339:113898. <https://doi.org/10.1016/j.scienta.2024.113898>.
- Carrasco G, Fuentes-Peñailillo F, Manríquez P, Rebolledo P, Vega R, Gutter K, Urrestarazu M. 2024. Enhancing leafy greens' production: Nutrient film technique systems and automation in container-based vertical farming. *Agronomy*. 14(9):1932. <https://doi.org/10.3390/agronomy14091932>.
- Chen S, Jin W, Liu A, Zhang S, Liu D, Wang F, Lin X, He C. 2013. Arbuscular mycorrhizal fungi (AMF) increase growth and secondary metabolism in cucumber subjected to low temperature stress. *Sci Hortic.* 160:222–229. <https://doi.org/10.1016/j.scienta.2013.05.039>.
- Diffenbaugh NS, Ashfaq M. 2010. Intensification of hot extremes in the United States. *Geophys Res Lett.* 37(15):15701. <https://doi.org/10.1029/2010GL043888>.

- Duan HX, Luo CL, Zhu Y, Zhao L, Wang J, Wang W, Xiong YC. 2024. Arbuscular mycorrhizal fungus activates wheat physiology for higher reproductive allocation under drought stress in primitive and modern wheat. *Eur J Agron*. 161:127376. <https://doi.org/10.1016/j.eja.2024.127376>.
- Faye A, Dalpé Y, Ndung'u-Magiroy K, Jefwa J, Ndoye I, Diouf M, Lesueur D. 2013. Evaluation of commercial arbuscular mycorrhizal inoculants. *Can J Plant Sci*. 93(6):1201–1208. <https://doi.org/10.4141/cjps2013-326>.
- Flores-Velazquez J, Mendoza-Perez C, Rubiños-Panta JE, Ruelas-Islas JdR. 2022. Quality and yield of bell pepper cultivated with two and three stems in a modern agriculture system. *Horticulturae*. 8(12):1187. <https://doi.org/10.3390/horticulturae8121187>.
- Fuentes-Peñailillo F, del Campo-Hitschfeld ML, Gutter K, Torres-Quezada E. 2025. Data-driven integration of remote sensing, agro-meteorology, and wireless sensor networks for crop water demand estimation: Tools towards sustainable irrigation in high-value fruit crops. *Agronomy*. 15(9):2122. <https://doi.org/10.3390/agronomy15092122>.
- Fuentes-Peñailillo F, Gutter K, Vega R, Silva GC. 2024. New generation sustainable technologies for soilless vegetable production. *Horticulturae*. 10(1):49. <https://doi.org/10.3390/horticulturae10010049>.
- Gruda NS. 2019. Increasing sustainability of growing media constituents and stand-alone substrates in soilless culture systems. *Agronomy*. 9(6):298. <https://doi.org/10.3390/agronomy9060298>.
- Grünfeld L, Skias G, Rillig MC, Veresoglou SD. 2022. Arbuscular mycorrhizal root colonization depends on the spatial distribution of the host plants. *Mycorrhiza*. 32(5–6):387–395. <https://doi.org/10.1007/s00572-022-01087-0>.
- Guo X, Wang P, Wang X, Li Y, Ji B. 2022. Specific plant mycorrhizal responses are linked to mycorrhizal fungal species interactions. *Front Plant Sci*. 13:930069. <https://doi.org/10.3389/fpls.2022.930069>.
- Hansen J, Sato M, Ruedy R. 2012. Perception of climate change. *Proc Natl Acad Sci U S A*. 109(37):E2415–E2423. <https://doi.org/10.1073/pnas.1205276109>.
- Harrison MJ. 2012. Cellular programs for arbuscular mycorrhizal symbiosis. *Curr Opin Plant Biol*. 15(6):691–698. <https://doi.org/10.1016/j.pbi.2012.08.010>.
- Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, Wilson GWT, Klironomos JN, Umbanhowar J. 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecol Lett*. 13(3):394–407. <https://doi.org/10.1111/j.1461-0248.2009.01430.x>.
- Kabir MY, Nambeesan SU, Bautista J, Díaz-Pérez JC. 2021. Effect of irrigation level on plant growth, physiology and fruit yield and quality in bell pepper (*Capsicum annuum* L.). *Sci Hortic*. 281:109902. <https://doi.org/10.1016/j.scienta.2021.109902>.
- Kakouridis A, Hagen JA, Kan MP, Mambelli S, Feldman LJ, Herman DJ, Weber PK, Pett-Ridge J, Firestone MK. 2022. Routes to roots: Direct evidence of water transport by arbuscular mycorrhizal fungi to host plants. *New Phytol*. 236(1):210–221. <https://doi.org/10.1111/nph.18281>.
- Kaya C, Ashraf M, Sonmez O, Aydemir S, Tuna AL, Cullu MA. 2009. The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. *Sci Hortic*. 121(1):1–6. <https://doi.org/10.1016/j.scienta.2009.01.001>.
- Kemble JM, Albornoz K, Bertucci MB, Bilbo TR, Jennings KM, Meadows IM, Melanson RA, Rodrigues C, Rudolph RE, Walgenbach JF, Wright-Smith HE, Wszelaki AL. 2025. 2025 Southeastern U.S. vegetable crop handbook. NC State Extension. <https://content.ces.ncsu.edu/southeastern-us-vegetable-crop-handbook>.
- Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking H. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*. 333(6044):880–882. <https://doi.org/10.1126/science.1208473>.
- Kokkoris V, Hamel C, Hart MM. 2019. Mycorrhizal response in crop versus wild plants. *PLoS One*. 14(8):e0221037. <https://doi.org/10.1371/journal.pone.0221037>.
- Luginbuehl LH, Oldroyd GED. 2017. Understanding the arbuscule at the heart of endomycorrhizal symbioses in plants. *Curr Biol*. 27(17):R952–R963. <https://doi.org/10.1016/j.cub.2017.06.042>.
- McNew J, Kathi S, Paudel P, Fontanier C. 2025. Effect of commercial mycorrhizae products on soilless media-grown basil yield and nutrient content in controlled environment conditions. *HortTechnology*. 35(5):745–747. <https://doi.org/10.21273/HORTTECH05703-25>.
- Milly PCD, Dunne KA. 2016. Potential evapotranspiration and continental drying. *Nat Clim Change*. 6(10):946–949. <https://doi.org/10.1038/nclimate3046>.
- Naumann G, Alfieri L, Wyser K, Mentaschi L, Betts RA, Carrao H, Spinoni J, Vogt J, Feyen L. 2018. Global changes in drought conditions under different levels of warming. *Geophys Res Lett*. 45(7):3285–3296. <https://doi.org/10.1002/2017GL076521>.
- Oliveira TC, Cabral JSR, Santana LR, Tavares GG, Santos LDS, Paim TP, Müller C, Silva FG, Costa AC, Souchie EL, Mendes GC. 2022. The arbuscular mycorrhizal fungus *Rhizophagus clarus* improves physiological tolerance to drought stress in soybean plants. *Sci Rep*. 12(1):9044. <https://doi.org/10.1038/s41598-022-13059-7>.
- Parniske M. 2008. Arbuscular mycorrhiza: The mother of plant root endosymbioses. *Nat Rev Microbiol*. 6(10):763–775. <https://doi.org/10.1038/nrmicro1987>.
- Portmann RW, Solomon S, Hegerl GC. 2009. Spatial and seasonal patterns in climate change, temperatures, and precipitation across the United States. *Proc Natl Acad Sci USA*. 106(18):7324–7329. <https://doi.org/10.1073/pnas.0808533106>.
- Roth R, Paszkowski U. 2017. Plant carbon nourishment of arbuscular mycorrhizal fungi. *Curr Opin Plant Biol*. 39:50–56. <https://doi.org/10.1016/j.pbi.2017.05.008>.
- Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, Pascale S, De Bonini P, Colla G. 2015. Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Sci Hortic*. 196:91–108. <https://doi.org/10.1016/j.scienta.2015.09.002>.
- Salomon MJ, Demarmels R, Watts-Williams SJ, McLaughlin MJ, Kafle A, Ketelsen C, Soupier A, Bücking H, Cavnarano TR, van der Heijden MGA. 2022. Global evaluation of commercial arbuscular mycorrhizal inoculants under greenhouse and field conditions. *Appl Soil Ecol*. 169:104225. <https://doi.org/10.1016/j.apsoil.2021.104225>.
- Smith SE, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: New paradigms from cellular to ecosystem scales. *Annu Rev Plant Biol*. 62:227–250. <https://doi.org/10.1146/annurev-arplant-042110-103846>.
- Thielicke M, Ahlborn J, Eichler-Löbermann B, Eulenstein F. 2023. On the negative impact of mycorrhiza application on maize plants (*Zea mays*) amended with mineral and organic fertilizer. *Microorganisms*. 11(7):Page :1663.

<https://doi.org/10.3390/microorganisms-11071663>.

Torres-Quezada E, Fuentes-Peñailillo F, Gutter K, Rondón F, Marmolejos JM, Maurer W, Bisono A. 2025. Remote sensing and soil moisture sensors for irrigation management in avocado orchards: A practical approach for water stress assessment in remote agricultural areas. *Remote Sens (Basel)*. 17(4):708. <https://doi.org/10.3390/rs17040708>.

Ullah F, Ullah H, Ishfaq M, Khan R, Gul SL, Gulfranz A, Wang C, Zhifang L. 2024.

Genotypic variation of tomato to AMF inoculation in improving growth, nutrient uptake, yield, and photosynthetic activity. *Symbiosis*. 92(1):111–124. <https://doi.org/10.1007/s13199-023-00961-5>.

Wang W, Shi J, Xie Q, Jiang Y, Yu N, Wang E. 2017. Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. *Mol Plant*. 10(9):1147–1158. <https://doi.org/10.1016/j.molp.2017.07.012>.

Williams EL, Abatzoglou JT. 2025. Climate change increases evaporative

and crop irrigation demand in North America. *Earths Future*. 13:e2025EF005931. <https://doi.org/10.1029/2025EF-005931>.

Xiao X, Liao X, Yan Q, Xie Y, Chen J, Liang G, Chen M, Xiao S, Chen Y, Liu J. 2023. Arbuscular mycorrhizal fungi improve the growth, water status, and nutrient uptake of *Cinnamomum migao* and the soil nutrient stoichiometry under drought stress and recovery. *J Fungi (Basel)*. 9(3):321. <https://doi.org/10.3390/jof9030321>.