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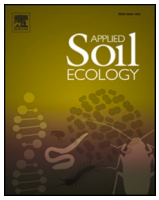

An assessment of twenty-three mycorrhizal inoculants reveals limited viability of AM fungi, pathogen contamination, and negative microbial effect on crop growth for commercial products

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ABSTRACT

Ensuring sustainable agriculture is crucial amidst global challenges, demanding effective methods to enhance soil health and nutrient cycling. Microbial inoculants, particularly arbuscular mycorrhizal (AM) fungi, offer promising solutions. However, concerns persist regarding the efficacy and quality control of commercial products. Past work assessing commercial inoculants have not controlled for fertilizers added to individual products when assessing product effects under typical use.

This study examines twenty-three mycorrhizal inoculants using conventions of organic production to shed light on differences between laboratory grown fungi, commercial products, and field soil. Employing a comprehensive approach, inoculants were assessed through spore enumeration, root infection potential, and crop growth response.

The results uncover significant shortcomings in many commercial products compared to laboratory grown fungi. Key findings include discrepancies of up to 100 % in reported propagule counts versus spore concentrations, insufficient root colonization by commercial inoculants, and contamination by fungal plant pathogens, particularly *Olpidium*, in products. Moreover, while laboratory grown fungi exhibited superior symbiotic relationships with host plants due to increased colonization abilities and crop benefit, commercial inoculants often failed to deliver significant growth benefits when fertilizers are controlled for.

These findings highlight the urgent need for improved standards and practices within the commercial inoculant industry.

1. Introduction

Enhancing sustainability remains a critical global challenge across ecosystems to ensure development within ecological limits. Beneficial microorganisms can facilitate nutrient cycling, improve soil health and stability, and aid phytoremediation and restoration. As a result, there is growing interest in reintroducing beneficial microorganisms into soils, particularly in organic agroecosystems where options for soil improvement may be limited. In response, microbial inoculants have emerged as a promising technology (O'Callaghan et al., 2022). Today, microbial inoculants are estimated to be a $10.3 billion dollar market (The Insight Partners, The Insight Partners, 2022).

Among microbial inoculants, arbuscular mycorrhizal (AM) fungi have garnered attention for their ability to enhance soil health and plant fitness (Basiru and Hijri, 2022), resulting in a 995 million USD world market for mycorrhizal inoculants (Mordor Intelligence, Mordor Intelligence, 2024). AM fungi form associations with 80 % of terrestrial plant species, including most crop species (Wang and Qiu, 2006), including annual (Hetrick et al., 1993; Jun and Allen, 1991; Vejsadova et al., 1993; Zhang et al., 2019) and perennial (Davies et al., 1993; McKenna et al., 2020) crops, such as grains, fruits, vegetables and oil seed crops. AM fungi are also important components of native ecosystems and many ecologically important plant species have been shown to benefit from mycorrhizal amendments in the field (Ortas, 2012; Tipton et al., 2022). Although AM fungi are commonly present in soils, AM fungal density, diversity and composition in soil may be altered by site history and soil disturbance given that land and chemical manipulations are known to alter fungal communities (Abbott and Robson, 1991; Jasper et al., 1991; Oehl et al., 2003; Ryan et al., 1994). Thus, fungal amendment via mycorrhizal inoculants could improve crop performance.

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The global commercialization of AM fungal inoculants faces significant challenges, including inconsistent field performance and quality control issues highlighted by studies where 50–84 % of tested inoculants failed to induce mycorrhizal root colonization (Salomon et al., 2022a; Tarbell and Koske, 2007). Another study used molecular methods and found that some inoculants contain none of the species on the product label (Vahter et al., 2023). Moreover, the presence of plant pathogens, including fungal pathogens in the genus *Olpidium*, can be detrimental to crop health (Hartwright et al., 2010; Lay et al., 2018) and pose an additional concern within the commercial inoculant industry. This pathogen was observed in a single inoculant in one study (Tarbell and Koske, 2007), however a comprehensive study assessing *Olpidium* has not been conducted. Inoculant viability and purity concerns necessitate the establishment of quality standards for commercial AM fungal inoculants. While some areas of the world such as Japan and the EU, have developed inoculant standards, there are no comprehensive standards for AM fungal inoculants in the US. Therefore, assessment of the viability and efficacy of commercial inoculants available in the US is needed.

The dissemination of strengths and weaknesses of AM fungal inoculants to consumers and producers faces hurdles due to limited communication between science and industry. In studies evaluating multiple commercial inocula, the omission of product names (Corkidi et al., 2004; Duell et al., 2022; Salomon et al., 2022a; Tarbell and Koske, 2007) hinders the applicability of results, limits the repeatability of studies, and may impede progress in addressing challenges within the inoculant production industry, which may source wholesale fungal isolates from similar sources. However, several researchers do report tested products (Faye et al., 2020; Wiseman et al., 2009). Additionally, testing of commercial AM fungal inoculants has often been confounded due to listed or unlisted fertilizers and other non-biotic amendments across products (Duell et al., 2022; Salomon et al., 2022a), which limit direct assessment of the biotic components of commercial products. That is, a separate control for each inoculant is required to de-confound potential fertilizers from AM fungal effects, which has not been done in previous tests of multiple commercial products. Furthermore, past studies of commercial inoculants have assessed inoculant efficacy in 80–100 % sand (Faye et al., 2013; Salomon et al., 2022a; Tarbell and Koske, 2007), potentially diminishing the relevance of findings to typical product use.

Here, the effectiveness of twenty-three AM fungal inoculants was studied, comparing academic laboratory grown fungi that are known to be symbiotic (Koziol and Bever, 2023; Koziol et al., 2023) with commercially available products and field soil. Inoculants were assessed for spore enumeration, root infection of AM fungi and fungal pathogens including *Olpidium*. A crop growth assay for two crop species was conducted using organic vegetable production standards under greenhouse settings. To assess the biotic contribution to plant response to inocula that varied in abiotic amendments, crop growth due to the active microbial effect was measured using replicates of unsterilized and sterilized inoculants. Findings were compared across studies, as well as how manufacturer's application recommendations related to inoculant viability and effects on crops. By comparing well-studied laboratory grown fungi with commercially available products, this study aimed to identify inoculant efficacy and inform the development of standardized quality control measures.

2. Methods

All experiments were conducted in the University of Kansas greenhouses in Lawernce, KS, USA.

2.1. Inoculant selection

Twenty-three amendments were selected for study (Table 1, for comprehensive product details see Supplement 1, Table S1). Sixteen commercially available arbuscular mycorrhizal (AM) fungal inoculants were chosen for analysis, with variations in product composition, microbial composition, and recommended application methods. Additionally, a vermicompost product was included for comparative purposes. The selection process involved consideration of product availability in US marketplaces and prioritization based on high rankings on the Amazon purchasing platform. Furthermore, two products were sourced from a local hardware store, while two were obtained as product samples from a vendor show. All selected products were confirmed to be non-expired, with expiration dates either listed as not having passed or unspecified. Products were stored at room temperature prior to use. Most commercial products were found to utilize calcined clay derived carrier media in powder or granular form.

Six laboratory grown cultures were selected (Table 1, Lab Grown). Laboratory grown cultures were produced occurring to previously established methods (Koziol et al., 2022a). Briefly, spores were collected from a remnant grassland prairie in Lawrence, KS, USA (39°02′48.2″N, 95°12′06.7″W) during 2012. Species of AM fungi were sorted microscopically, and single species cultures were grown with a *Sorghum* × *drummondii* hybrid as host plants on steam sterilized field soil:sand. Cultures were propagated yearly, and the sixth generation of these cultures was used. Cultures for this study were grown in 2019 and stored dry at 4 °C for 13 months prior to use in this study. Selected species encompassed those commonly found in commercial inoculants (e.g., *Funneliformis mosseae* and *Rhizophagus irregularis*), species that are rarely or never found in commercial inoculants (e.g., *Ambispora* sp.), and others. Additionally, a mixture was created by combining equal proportions of all six individual laboratory grown AM fungi (Table 1, Lab Species Mix). Field soils were collected from an organic tomato farm the day before inoculation and spore assessment (Juniper Hill Farms, 39°01′45.3″N, 95°12′41.2″W).

2.2. Spore propagule assessment

To quantify the concentration of AM fungal spores in each inoculum, four samples of each inoculum were weighed before undergoing spore wet extraction. Commercial products were evaluated at 1–2 times the recommended application rate, while laboratory grown cultures were assessed in 50 cm^3 (exact volumes can be found in Table S2). Spores were extracted with water using 1 mm and 0.038 mm sieves, followed by drip immersion onto a 60 % sucrose solution and subsequent centrifugation for 1 min. The supernatant was washed on a 0.038 mm sieve before being transferred to a petri dish covered with a 97-grid plate. Samples were homogenized with water before spore enumeration was conducted using a stereo dissecting scope at 40× magnification on a subset of each plate. An estimated number of spores per plate was extrapolated to calculate spores per gram for each sample. Species differentiation and spore viability were not performed. The percentage of propagules that were spores was calculated using the manufacturer's reported propagule number and spore count enumeration. The spore number per gram was log-transformed and analyzed using a general linear model in SAS (SAS, 2013), with block and inoculum as predictors.

2.3. Inocula infection viability

A comprehensive Mean Infection Potential (MIP) experiment was conducted, encompassing all inocula and a non-inoculated control. Conetainer pots (150 cm^3) were 90 % filled with autoclaved sand:soil. Inoculants were applied and then the remainder of the pot was filled with the sterile soil mixture before being planted with a 2 cm tall *Sorghum* seedling. Each of five replicates were sorted into fully randomized blocks. Three replicates of KM, GW, and NLA inoculants were used due to inoculant availability. The recommended application rate for each product was used, ranging from 0.6 g to 6.9 g per inoculant (Table 1). An estimated number of AM propagules applied to each pot was calculated using the manufacturer's specifications and the spore count for

Table 1
Inoculants utilized in the crop growth assay, colonization (MIP), and spore enumeration studies are marked with a 1 if included and a 0 if not included in each analysis. Propagule number is based on manufacturers' labels for commercial products and spore enumeration for field soil and laboratory grown inoculants.

								Crop Growth Study		Colonization MIP		
Included in Growth Assay	Included in MIP	Included in Spore Enumeration	Product Type	Product or Inoculant Name	Abbreviation	Listed Species	AMF prop/ g or mL	Application Weight (g)	Estimated AMF Propagules Applied	Application Volume (mL)	Estimated Application Weight (g)	Estimated AMF Propagules Applied
1	1	1	Commercial	Great White Premium Mycorrhizae	GW	9	387.0	5	1935	5	3.30	1277
1	1	1	Commercial	Xtreme Gardening Mykos	XG	1	300.0	5	1500	2.5	1.76	527
1	1	1	Commercial	Plant Probiotics	PP	4	5.6	5	28	1.25	0.86	5
1	1	1	Commercial	Green Eden Endo Boost Pro	GE	7	104.8	5	524	5	3.98	416
1	1	1	Commercial	Big Foot Mycorrhizae Granular	BF	1	66.0	5	330	5	6.92	457
1	1	1	Commercial	Root Naturally Granular Endo Mycorrhizae	RN	4	132.2	5	661	2.5	1.72	227
1	1	1	Commercial	Root Magic Mycorrhizae +	RM	4	284.0	5	1420	2.5	1.30	370
1	1	1	Commercial	Myco Bliss	MB	5	1000.0	5	5000	5	3.10	3097
1	1	1	Commercial	Mikrobs	M	4	28.0	5	140	1.25	0.86	24
1	1	1	Commercial	Dynomyco Premium Mycorrhizal Inoculant	D	2	900.0	5	4500	5	4.78	4303
1	1	1	Commercial	Wildroot Organic	WO	9	446.0	5	2230	1.25	0.60	267
1	1	0	Commercial	Earth Science Earthworm Castings	ES	0	0.0	5	0	15	7.86	0
1	1	0	Commercial	Happy Frog	HF 5	4	0.0	5	0.01	15	2.54	0.04
1	0	0	Commercial	Happy Frog	HF 50	4	0.0	50	0.08	NA	NA	NA
1	1	0	Commercial	Promix Organics	PMO 5	1	1.0	5	5	15	1.95	2
1	0	0	Commercial	Promix Organics	PMO 50	2	1.0	50	50	NA	NA	NA
0	1	1	Commercial	King of Mycorrhizae	KM	4	175.0	NA	NA	5	4.00	699
0	1	1	Commercial	New Life Agriculture/ Microbial Solutions	NLA	3	16,291.0	NA	NA	5	5.00	81,455
1	1	1	Lab Grown	*Gigaspora gigantea*	*Gi. gigantea*	1	1.3	6.2	8	5	6.20	8
1	1	1	Lab Grown	*Rhizophagus irregularis*	*R. irregularis*	1	117.2	6.2	727	5	6.20	727
1	1	1	Lab Grown	*Funnilformis mosseae*	*F. mosseae*	1	6.9	6.2	43	5	6.20	43
1	1	1	Lab Grown	*Rhizophagus clarus*	*R. clarus*	1	10.2	6.2	63	5	6.20	63
1	1	1	Lab Grown	*Ambspora sp.*	*Ambispora sp.*	1	15.0	6.2	93	5	6.20	93
1	1	1	Lab Grown	*Glomus mortonii*	*Gl. mortonii*	1	9.3	6.2	57	5	6.20	57
1	1	0	Lab Grown	Lab Species Mix	Lab Species Mix	1	26.6	6.2	165	5	6.20	165
1	1	1	Field Soil	Field Soil	Field Soil	NA	6.0	6.2	37	5	6.20	37

laboratory grown cultures. Estimated propagule application ranged from <1 to 81,455 AM fungal propagules per pot (Table 1, Fig. 1A). Plants were grown for 60 days beginning October 1st, 2020, with daily watering. Upon harvest, soil was gently washed from plant roots, and roots were subsampled at approximately 3–4, 8–9, and 13–14 cm depths. Roots were stained with Trypan Blue to assess root colonization (McGonigle et al., 1990). Because colonization was extremely low for some inoculants, the presence of AM fungal structures (including hyphae, arbuscules, and vesicles) and pathogens, identified as an *Olpidium* sp., were assessed on each of ten randomly selected 1 cm root fragments. The proportion of roots colonized with the various microbial structures was analyzed using a general linear model with block and inoculant as predictors (SAS, 2013).

2.4. Crop growth

Crop growth responses to inoculation were evaluated for two commonly cultivated cool-season species: lettuce (Black Seeded Simpson, *Lactuca sativa,* NK Lawn & Garden*)* and carrot (Danvers, *Daucus carota,* NK Lawn & Garden), utilizing 4-in. peat pots (500 cm^3). Pots were filled 90 % with Ocean Forest Potting Soil (FoxFarm, Arcata, CA, USA), which is a sandy loam with sphagnum peat moss and other amendments. This non-AM fungi containing mixture was chosen due to its typical use in organic crop production by an organic farm consultant at Juniper Hill Farms. Inoculants were applied and then the remainder of the pot was filled with the potting soil before a 1 cm seedling was planted in each pot. We applied approximately 5-6 g of each inoculant, with the two potting soil inoculants having both a low 5 g and a high 50 g application rate (Table 1). To mitigate potential confounding effects from fertilizers and other components present in the different inoculants, three control groups were included: 1) no inoculant added, and 2) 5 g of autoclaved sterilized inoculant was added for each product, totaling 24 unique inoculant control types and 3) a mixture of all sterilized inoculants was added. Each combination of crop species, inoculant type, and control inoculant was replicated five times in a fully randomized block design. The exception was GW inoculant, which had four replicates with carrot only due to inoculant availability. Pots were grown under ambient light with daily watering for 8 weeks beginning in October of 2020. At the time of harvest, aboveground biomass was collected from all plants, and roots from two replicates were carefully washed. One was used to calculate root mass, and another was subsampled and analyzed for mycorrhizal infection using the previously described methods in the MIP analysis. Total crop biomass was log transformed prior to analysis to ensure normality. A mixed generalized linear model was used to assess growth, with replicate, and all interactions of inocula viability (as packaged or sterilized via autoclaving), inocula, and crop species as predictors (SAS, 2013).

Because nutrient content differences among various inocula were expected, the effect of product media and nutrient addition via inocula was assessed and is henceforth referred to as the Fertilizer Effect. The Fertilizer Effect was calculated for each inoculant within each block as follows:

$$\text{Fertilizer Effect (FE)} = \frac{\text{plant biomass with each sterilzed inoculant}}{\text{plant biomass in the no inocula added control}}$$

To conduct a formal test of the microbial contribution to growth, the effect of product fertilizer content was removed by assessing growth with active inocula (as packaged, unsterilized) relative to that same inoculant when sterilized. The active microbe effect was calculated within block as:

$$\text{ActiveMicrobeEffect}\left(\text{AME}\right) = \ln\left(\frac{\text{plant biomass with each unsterilized inoculan}}{\text{plant biomass with same sterilzed inoculant}}\right)$$

Both AME and FE response variables were analyzed using a generalized mixed model (SAS, 2013), with block, inocula, crop species, and inocula by crop species interactions as predictors. Five samples were removed from AME or FE calculations due to crop death (IDs 5, 20, 26, 78, 231). The roots of two samples were mislabeled (ID 425 and 452), and thus only shoots were used to calculate AME and total crop growth for the paired samples in those blocks.

2.5. Correlations

To examine relationships between crop growth and inoculant properties across studies, the log-transformed means from model outputs

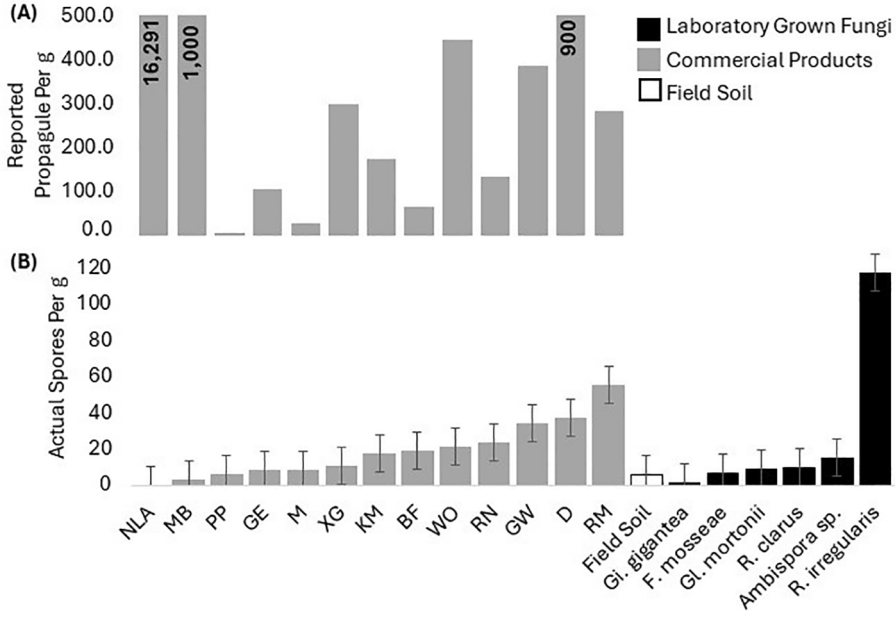

Fig. 1. (A) Reported propagules per gram on product labels. Commercial product propagule count may include spores, hyphae, and infective root counts. Three propagule counts are not depicted in this figure due to sizing and is instead highlighted in bold text superimposed on the corresponding bar. (B) The actual spore count per gram for each inocula, determined via wet sieved spore extraction. The bars represent LS means and error bars indicate standard error from model outputs.

were assessed in a correlation analysis using Proc Corr (SAS, 2013). The variables included were spore number per gram, the Active Microbe Effect (AME), crop growth in unsterilized inoculum for lettuce and carrots, the Fertilizer Effect (FE), and the proportion of roots infected with hyphae, arbuscules, vesicles, and *Olpidium*. Additionally, the reported propagules per gram from both commercial products and laboratory grown inoculants (spore counts) were incorporated into the analysis after log (1+ count) transformation. This inclusive approach aims to elucidate potential relationships among these key variables.

3. Results

The reported AM fungal propagule count for commercial products exhibited a wide range, spanning from 5.6 to 16,291 propagules per gram or mL (Fig. 1A). The assessed spore concentration significantly varied among inoculants ($F_{19,79} = 13.9, p < 0.0001$), which ranged from 0 to 55 spores per gram for commercial inoculants and from 1 to 117 spores per gram for laboratory grown inoculants (Fig. 1B, Fig. S1A). While spore enumeration closely matched reported propagule count for one commercial inoculant, all others showed discrepancies ranging 72 % to 100 % (Fig. S1B, $F_{12,51} = 10.3$, $p < 0.0001$). Averaging the mean enumerated spore count and reported propagule counts across all commercial inoculants, spores were found to represent <2 % of the reported propagules. Given that none or few roots were observed among commercial inoculants, this would require that 98 % of reported propagules were hyphal fragments.

The Mean Infection Potential (MIP) root analysis was used to assess fungal colonization ability across all types of propagules. MIP indicated significant variability in AM fungal hyphae colonization ($F_{24, 114} = 4.56$, $p < 0.001$) and arbuscule colonization ($F_{24, 114} = 2.1$, $p = 0.007$) among the inoculants. Eight commercial products showed no AM fungal colonization in any pot, despite applications of up to 81,455 reported propagules of AM fungi (Fig. 2A & B). Non-inoculated roots demonstrated no observable AM fungal colonization (Fig. S2). The laboratory grown *R. irregularis* inoculant, a fungus listed in almost all tested commercial inoculants, infected roots (Fig. S2). AM hyphal colonization was notably low in the commercial inoculants, with the highest being around 6 %, lower than field soil at 11 % (Fig. 2B). In contrast, inoculation with most laboratory grown inocula resulted in consistent hyphal colonization, ranging from 11 % with *F. mosseae* to 49 % with *R. irregularis* (Fig. 2B). Arbuscular colonization was observed in field soil and with all laboratory grown fungi, but only one commercial product resulted in arbuscules (Fig. S3).

Five commercial products and the field soil were consistently contaminated with a fungal pathogen ($F_{24, 114} = 9.76$, $p < 0.0001$), identified as an *Olpidium* species, ranging from 33 % to 86 % colonized (Fig. 2C). Product GW and laboratory grown *Gi. Gigantea* each had one replicate colonized by *Olpidium*, albeit to a lesser extent (a few spores, compared to a plethora in the other commercial products, Fig. 3A–G). No *Olpidium* was observed in the controls or in any other inoculants.

3.1. Crop growth among inocula

Crop growth varied by crop species ($F_{1, 381} = 273.65$, $p < 0.0001$), inoculant type ($F_{24, 281} = 9.03$, $p < 0.0001$), and their interaction ($F_{23, 381} = 2.44$, $p = 0.0003$), with some inoculants increasing crop growth relative to the control (Fig. S4). This was strongly driven by the fertilizer effect (FE), which was highly dependent on inoculant ($F_{24, 189} = 4.45$, $p < 0.0001$). Interestingly, the overall effect of inoculants being sterilized did not impact overall crop growth ($p = 0.71$), which aligns with the findings from the MIP analysis indicating that many inoculants were unviable. However, some inoculants affected crops differently when sterilized (Fig. S4, $F_{24, 381} = 1.62$, $p = 0.035$).

The Active Microbe Effect (AME) was conducted as a formal test of how microbes affected crops, where the effect of product fertilizer content was removed using sterilized inoculants as controls. Inoculant emerged as the most influential predictor of crop response to the AME (Fig. 4, $F_{24, 187} = 2.76$, $p < 0.0001$) and crop species' AMEs were similar among inoculants ($p = 0.23$). Among the twenty-four treatments assessed, only seven inoculants showed significant differences from

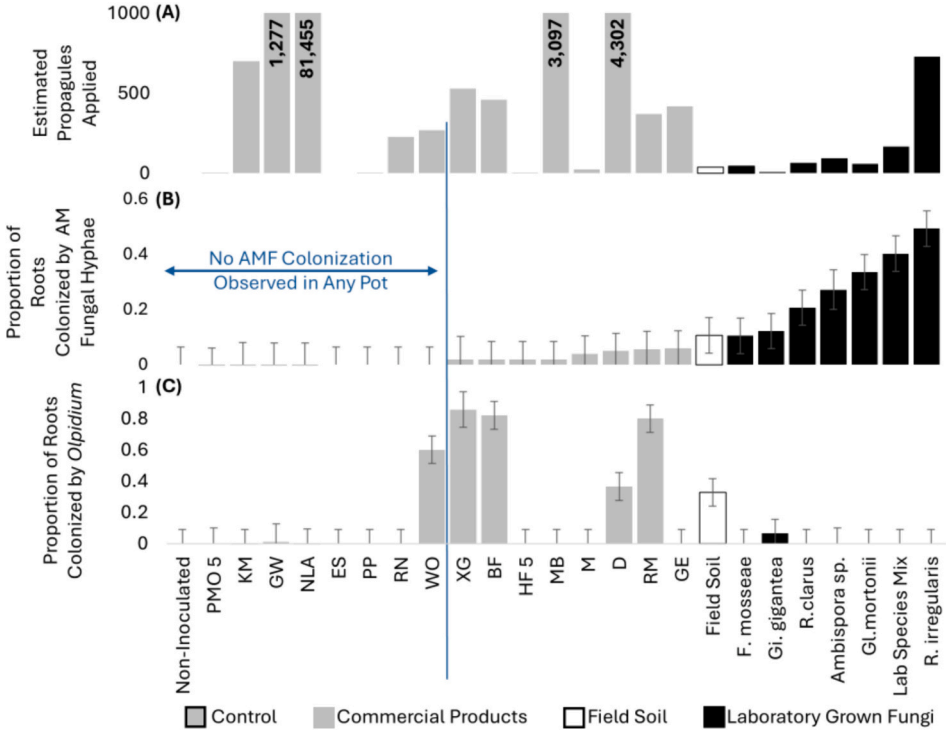

Fig. 2. (A) Estimated propagules applied on *Sorghum* roots in the MIP based on the manufacturer's product labels or spore counts. The propagule count for four inoculants is not depicted due to sizing and is instead highlighted in bold text superimposed on the corresponding bar. The proportion of roots colonized by (B) AM fungal hyphae and (C) the fungal pathogen *Olpidium*. Bars in B & C represent LS means and standard error from model outputs.

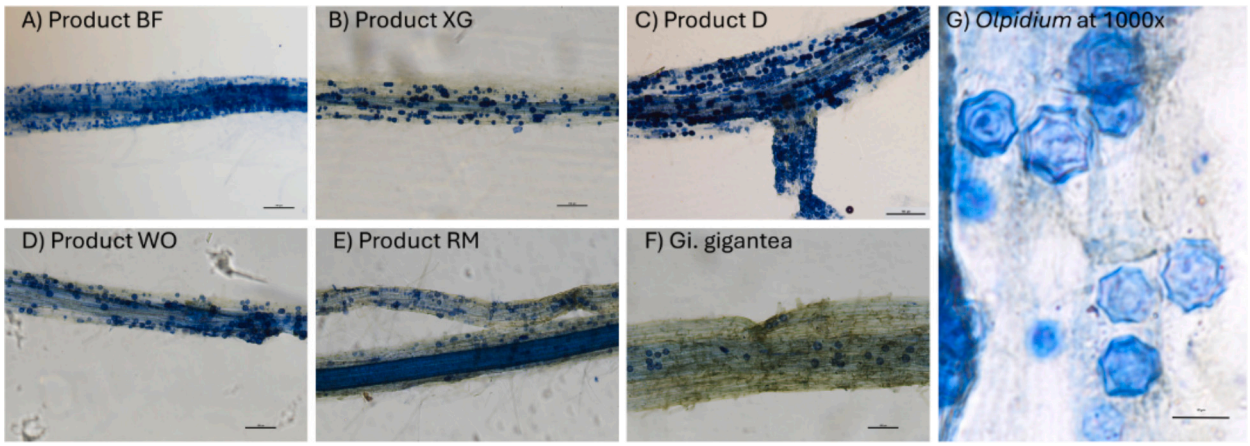

Fig. 3. *Olpidium* sp. root colonization at 100× (100 μm scale) in (A-E) commercial products and (F) in a laboratory grown inocula. (G) *Olpidium* spores at 1000× (10 μm scale bar).

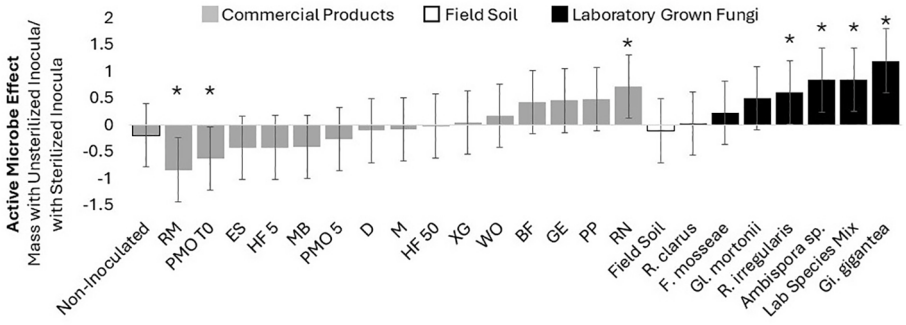

Fig. 4. The Active Microbe Effect (AME) on crop growth was overall positive for laboratory grown fungi and negative for commercial products. Bars represent LS means and 95 % CI from model outputs. Stars represent inoculants whose effect on crop growth was significantly different when the inoculant was active (non-autoclaved) versus a sterilized control of that inoculant.

zero. Interestingly, two commercial inoculants inhibited crop growth when alive, while five inoculants, including one commercial and four laboratory grown fungi, improved crop growth when microbes were alive. After removing the effects of fertilizer, laboratory grown fungi had an average active microbe effect of +0.60 while commercial inoculants inhibited crop growth by an average of −0.06. Four out of five *Olpidium* contaminated inoculants had detrimental AME effects on the growth of lettuce, carrot, or both.

3.2. Correlations

The inocula Fertilizer Effect (FE) effect was strongly negatively correlated with fungal root infection (Fig. 5A) and the Active Microbe Effect (AME) (Fig. 5B). This indicates that inoculants with more fertilizer had less root colonization. Unsurprisingly, there was a strong positive correlation between the FE and crop growth (Fig. 5C). Across the growth and MIP studies, a positive correlation was observed between the proportion of roots infected with arbuscular mycorrhizal (AM) fungi and crop sensitivity to the AME (Fig. S5). Interestingly, the manufacturer's reported propagules per gram was not correlated with spore

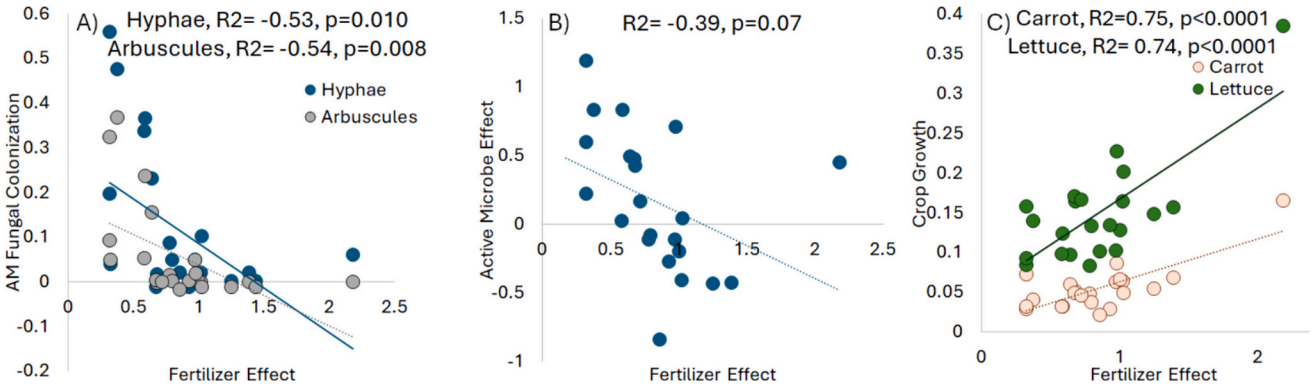

Fig. 5. The fertilizer effect showed a negative correlation with A) AM fungal colonization and B) the Active Microbe Effect, while displaying a positive correlation with C) overall crop growth.

concentration, root colonization by AM fungi, AME, or crop growth (Fig. S6). Moreover, spore count was not correlated with hyphal infection, as several products with spores did not result in observable AM fungal infection, and inoculants with both high and low spore concentrations had low viability (Figs. S7 & S8).

4. Discussion

The benefits of AM fungal inoculation have been demonstrated across ecosystems, driving interest and investment in microbial inoculants for enhancing plant growth and soil health. This study evaluated twenty-three inoculants for their AM fungal concentration, symbiotic root associations, and their effect on lettuce and carrot growth in organic agriculture. While laboratory grown AM fungi showed superior symbiotic associations with host plants, commercial products demonstrated product label disparities, limited symbiotic benefits to host, and the presence of pathogenic fungi. These findings reinforce the need for improved quality control in the commercial inoculant industry.

4.1. Inoculant root infectivity and effect on crop growth

Spore assessments found that spore concentration was 71 %–100 % lower than the reported propagule count on manufacturers product listings for all but 1 product. Past studies have found that some commercial products contain many fewer spores than the listed propagule count (Salomon et al., 2022a; Tarbell and Koske, 2007). It should be noted that “propagule” can describe any viable fungal material, including spores, and infective roots or hyphal segments. The mean infection percentage (MIP) was used to assess viability and infection of all types of propagules. After applying, in some cases >80,000 propagules based on the manufactures labels, 50 % of commercial products resulted in no observable AM fungal infection in three crops, sorghum, carrot, or lettuce, and the greatest infection potential of a commercial inoculant was around 10 % in the MIP. Past work assessing US (Tarbell and Koske, 2007), Kenyan (Faye et al., 2013), and European and Australian (Salomon et al., 2022a) commercial AM fungal products found that most inoculants result in hyphal colonization of <10 %. Although previously suggested that application rates may need to be five to ten times greater than manufacturer recommendations (Tarbell and Koske, 2007), others have found that twenty to forty times recommended application rates may still not result fungal colonization, indicating that some products may not be viable at any application rate (Wiseman et al., 2009).

Considering crop growth, lettuce and carrot responded differently to the individual inoculants. This effect was driven by each product's fertilizer effect (FE), which was strongly positively correlated with growth for both crops, and negatively correlated with AM fungal colonization and benefit from microbes. Past work has shown that inoculants contain labeled or unlabeled nutrient additions in products (Duell et al., 2022; Salomon et al., 2022a), making comparisons across inoculants challenging in past work. A novel aspect of this study was use of the active microbe effect (AME), which was calculated using paired sterile controls for each inoculant to remove abiotic contributions to crop growth, such as fertilizers. Inoculants that had higher AME also had greater root colonization. Overall, the AME for thirteen commercial inoculants was benign, while two inhibited crop growth and one promoted crop growth. In contrast, all laboratory grown inoculants promoted crop growth via the active microbe effect, with four being significantly greater than controls. Future work should assess the AME under field conditions. Past work has shown that response to mycorrhizal inoculants in the greenhouse often predicts responses observed in the field (Koziol et al., 2019; Koziol et al., 2022b; Pringle and Bever, 2008).

4.2. What is a propagule? The effect of manufacturing practices on product viability and crop benefit

The lack of symbiotic ability or viability found in some commercial products may be due to the manufacturing practices of producers. Mycorrhizal products can be manufactured using genetically transformed plant roots under in vitro cultivation (Cranenbrouck et al., 2005), offering scalability for commercial production but potentially compromising their symbiotic capabilities (Calvet et al., 2013; Kokkoris and Hart, 2019). Supporting this hypothesis, only one commercial inoculant exhibited arbuscular formation in the MIP, and only one commercial inoculant was beneficial when the active microbial effect was calculated. In contrast, laboratory grown cultures, which use in vivo cultivation and a soil derived media, produced arbuscules across all seven inoculants, with four significantly improving crop growth via the active microbe effect, potentially highlighting the superior symbiotic benefit of AM fungi produced in vivo. The extent to which in vivo versus in vitro processes are used in commercial AM fungal inoculants is often undisclosed.

A second consideration is the use of granular or powdered calcined clay and zeolite (both often used as cat litter) as a carrier media for many mycorrhizal inoculants, as these media are industrial desiccants that may harm hyphal propagules. In this study, it was extrapolated that around 98 % of propagules listed on commercial products were hyphal fragments. AM hyphae are delicate and aseptate, making them vulnerable to desiccation and disturbance, thereby potentially reducing product viability. Past work has shown that while all tested AM fungal species were able to colonize from spores, 43 % and 50 % of species were unable to colonize via freshly collected roots or hyphal fragments, respectively (Klironomos and Hart, 2002), and evidence that disturbance and desiccation of AM fungal hyphae can render hyphae nearly or totally non-viable far predates the mycorrhizal inoculant boom (Bellgard, 1992; Jasper et al., 1989). Given that hyphal fragments are unlikely to remain viable in inoculants at the time of product use, we recommend removing hyphal concentrations in propagule counts on product listings, as use of the word “propagule” necessitates viability. AM fungal spores should be the primary source of propagule count, as spores are more likely to survive disturbance and desiccation during manufacturing processes, as is evident from the laboratory grown fungi in this trial, which were desiccated for around 13 months prior to use. In 2020, two US regulatory bodies declared that hyphae are no longer recognized as endomycorrhizal propagules (CDFA, 2020). It should be noted that all products tested in this experiment were purchased after these definitions were established.

4.3. Product contamination

Five commercial inoculants had prominent levels of colonization by an *Olpidium* species, which was determined to be *Olpidium brassica*, an obligate root fungal parasite recognizable due to its distinct shape. *Olpidium* species are known as fungal pathogens and vectors for crop affecting viruses, including the crops used in this study, fava bean, tobacco, melon, canola, brassicas, and others (Hartwright et al., 2010; Meresa and Gebremedhin, 2020; Tomlinson et al., 1983). Because *Olpidium* was never observed in the non-inoculated controls, and because these inoculants were spatially distributed and observed to contain *Olpidium* across replicates, it can be concluded that the inoculants were contaminated prior to being used in this study. Overall, 4/5 products frequently colonized by *Olpidium* demonstrated a detrimental effect on lettuce or carrot growth when microbes were active. Past work has shown that 1 in 8 US sourced commercial inoculants was contaminated with *Olpidium* (Tarbell and Koske, 2007), whereas this study found 5 in 16 US sourced products were contaminated, potentially indicating an increasingly problematic pattern in the largely unregulated microbial inoculant industry. The extent of this concern globally is unknown.

4.4. Conclusion on the value of viable symbionts

Contrary to what was observed with commercial inoculants, the laboratory grown AM fungi, which also included the predominant fungus in most commercial products, *R. irregularis*, showed strong symbiotic relationships with plants and a positive active microbe effect on crop growth. Overall, the laboratory grown fungal inoculants highlight the potential for AM fungal inoculants to enhance crop growth in organic agriculture. However, commercial products were found to suffer from product label inaccuracies, low viability, and pathogen contamination. Commercial products had fewer symbiotic root structures, and the inocula was more likely to harm than help crop growth after the effects of fertilizers were removed. These findings contribute to the growing body of work highlighting the challenges facing the global commercialization of AM fungal inoculants, emphasizing the need for standardized quality control and improved communication between science and industry to enhance microbial inoculant efficacy for agricultural sustainability. Although previous work has suggested frameworks for cultivation and product standard testing (Salomon et al., 2022b; Vosátka et al., 2012), these recommendations differ and have not been adopted on a global scale.

The following supporting information can be downloaded upon article publication, including all data and SAS codes, and figures S1–8. Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105559.

CRediT authorship contribution statement

Liz Koziol: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Terra Lubin:** Writing – review & editing, Investigation, Formal analysis. **James D. Bever:** Writing – review & editing, Funding acquisition, Formal analysis.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author LK used ChatGPT to correct text for grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

Liz Koziol reports financial support was provided by National Science Foundation. James D Bever reports financial support was provided by National Science Foundation. Terra Lubin reports financial support was provided by National Science Foundation. Liz Koziol reports a relationship with MycoBloom that includes: equity or stocks, speaking and lecture fees, and travel reimbursement. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data presented in this study will be available in the supplement upon manuscript publicaiton.

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