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RESEARCH ARTICLE

Linking soil health to human health: Arbuscular mycorrhizae play a key role in plant uptake of the antioxidant ergothioneine from soils

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Societal Impact Statement

Evidence has emerged that the antioxidant ergothioneine may be important in preventing many inflammatory diseases in humans. However, ergothioneine is not produced by humans or plants and is only made by fungi and some bacteria in soils. As such, humans get ergothioneine from eating fungi (mushrooms) or plants that take it up from the soil. In this study, we found that growing plants with beneficial fungi called arbuscular mycorrhizal fungi increased the amount of ergothioneine in plant tissues. This suggests that promoting agricultural practices that maintain healthy populations of beneficial soil fungi may improve the nutritional quality of crops.

Summary

- The amino acid ergothioneine (ERGO) has recently gained attention as an antioxidant that benefits human health. ERGO is produced by fungi and mycobacteria in soils and is acquired only from diet. The mechanism by which ERGO is transferred from soil to plant is unknown. Recent work has shown that tillage reduces the amount of ERGO in crops. As tillage also reduces arbuscular mycorrhizal fungi (AMF) populations, we examined the relationship between AMF and plant ERGO uptake.
- We grew asparagus, black beans, wheat, and oats with a variety of single and mixed species AMF inocula and compared ERGO levels of these plants to plants that were uninoculated.
- Mycorrhizal inoculation enhanced ERGO content across all plants. There was a positive correlation between AMF colonization level and plant ERGO content.
- AMF appear to be important mediators of plant ERGO uptake. Future research is needed to identify the mechanism that leads to higher ERGO in plants colonized by AMF in order to promote farming practices that enhance AMF populations and increase crop ERGO concentration in field settings.

KEYWORDS
antioxidants, arbuscular mycorrhizae, ergothioneine, liquid chromatography–tandem mass spectrometry, nutrition, plant health, soil health

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1 | INTRODUCTION

Ergothioneine (herein ERGO) is a naturally occurring amino acid that has recently gained attention as a potent antioxidant and anti-inflammatory, and as such, it has been labeled as a potential "longevity vitamin" and "powerhouse nutrient" (Ames, 2018; Beelman et al., 2020). In humans, ERGO is derived exclusively from diet and is absorbed via the highly selective ERGO transporter during digestion where it accumulates in tissues that are susceptible to high levels of oxidative stress such as blood, eye lens, liver, bone marrow, and seminal fluid (Beelman et al., 2020; Cheah et al., 2016; Gründemann et al., 2005; Melville, 1959; Paul & Snyder, 2010; Shires et al., 1997). ERGO consumption has been linked to a decreased risk of heart and cerebrovascular disease (Smith et al., 2020; Wu et al., 2021). In addition, lower life expectancy and higher mortality from neurological disorders in the United States compared with several European countries have been linked to less ERGO consumption (Beelman et al., 2020; Ramirez-Martinez et al., 2016).

Although ERGO is acquired solely from the diet, it is not synthesized by plants, is not detected in all plants, and there appears to be high variation in ERGO levels in raw foods that have been tested thus far (Ey et al., 2007; Halliwell et al., 2018). In fact, ERGO is synthesized exclusively by fungi, mycobacteria, and cyanobacteria and is therefore consumed only by eating the fruiting bodies of fungi (mushrooms) or by eating plants that have acquired ERGO from soil or soil microorganisms (Borodina et al., 2020; Ey et al., 2007; Genghof, 1970; Genghof & Damme, 1964; Halliwell et al., 2018; Kalaras et al., 2017; Narainsamy et al., 2016). However, the mechanism by which ERGO is transported from the soil into plants is unknown. Therefore, there is a need to understand the processes that lead to ERGO uptake by plants to promote or develop agricultural practices that increase the amount of ERGO in major crops.

Recent research has linked farming tillage practices to the ERGO content of corn (*Zea mays*), soybean (*Glycine max*), and oats (*Avena sativa*) (Beelman et al., 2021). Specifically, this work has shown that the ERGO content of all three crops was the highest under no till practices and declined with increased tillage intensity (Beelman et al., 2021). One possible mechanism linking soil disturbance to reductions in ERGO uptake are well-documented declines in the population of arbuscular mycorrhizal fungi (AMF) under long-term tillage (Bowles et al., 2017; Brito et al., 2012; Dai et al., 2015; Frey et al., 1999; Kabir, 2005). AMF are symbiotic fungi that associate with over 80% of plants (including crops) and enhance plant access to nutrients and water, which the fungi exchange for photosynthetically derived carbon (C) (Read & Perez-Moreno, 2003). Therefore, it is possible that AMF play a role in the plant uptake of ERGO from soils.

To test the association of crop ERGO and AMF colonization, we grew asparagus, black beans, wheat, and oats in a variety of media with treatments that included uninoculated, single species AMF inoculation, and mixed species AMF inoculation. Mycorrhizal root colonization was measured across AMF treatments and compared with the ERGO concentration of plant tissue at harvest.

2 | METHODS

2.1 | Inoculum preparation

Individual mycorrhizal species accessions were obtained from the International Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) at West Virginia University in Morgantown, WV, USA or in the case of *Septoglomus constrictum* from the AMF collection at the USDA Eastern Regional Research Center (ERRC) in Wyndmoor, PA, USA (Table 1). The commercial product, AGTIV® (*Rhizophagus irregularis*), was provided by Premier Tech Horticulture (Riviere-du-Loup, Quebec, Canada). AMF were maintained in association with Bahia grass (*Paspalum notatum* Flügge) grown in a combination of sand, sterile soil, vermiculite, and turface (herein SSVT; 1:0.75:1:0.75 v/v) (Douds et al., 2010). A mixed species inoculum representing a native AMF community was propagated by preparing a 1:10 dilution of agricultural field soil from organically managed vegetable plots at the Rodale Institute in Kutztown, PA, USA (herein natural community). For the asparagus experiment, whole soil inoculum from SSVT pot cultures was used directly for inoculation, and for the black bean, wheat, and oat experiments, the increased volume of inoculum needed was produced one growing season ahead in 5-gal grow bags and overwintered outdoors prior to use as in Douds et al. (2010). To prepare inoculum, the aboveground biomass of the Bahia grass plants was removed, and root tissue was cut into ~1-cm lengths and reincorporated into SSVT. In all experiments, plants grown under control

Species	Accession ID	Source	Site of origin
Claroideoglomus claroideum	PA104A	INVAM	Kutztown, PA, USA
Claroideoglomus etunicatum	PA127B	INVAM	Pennsylvania, USA
Funneliformis mosseae	PA127A	INVAM	Pennsylvania, USA
Gigaspora rosea	FL105	INVAM	Florida, USA
Scutellospora calospora	PA103A	INVAM	Pennsylvania, USA
Septoglomus constrictum	LGEO	ERRC	Kutztown, PA, USA
Rhizophagus intraradices	QE102	INVAM	Quebec, Canada
Rhizophagus irregularis	ON205B	INVAM	Ontario, Canada
Rhizophagus irregularis—AGTIV®	N/A	Premier Tech®	N/A

TABLE 1 Fungal spore isolates used to propagate single-species arbuscular mycorrhizal fungi (AMF) inoculants. INVAM is the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi, West Virginia University, Morgantown, WV, USA. ERRC is USDA-ARS Eastern Regional Research Center, Wyndmoor, PA, USA. AGTIV is the name of a commercial product produced by Premier Tech Horticulture (Riviere-du-Loup, Quebec, Canada). N/A indicates not applicable.

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conditions were "mock inoculated" with sterilized (autoclaved) uninoculated Bahia grass grow bag media. This was to ensure that measured plant responses were not the result of the addition of grow bag media but were driven by mycorrhizal inoculation.

We chose to inoculate black beans and asparagus with all the AMF species propagated in our lab that met both the number of spores needed for each trial and exhibited no cross-contamination as determined by visual inspection of spores at the time of planting. For this reason, the black bean trial included three additional AMF inoculations compared with the asparagus trial. We chose to use all available species based on our previous work that found a range of colonization efficiencies across AMF and host species in corn (*Z. mays*) and butternut squash (*Cucurbita moschata*) (Carrara & Heller, 2022). Wheat and oat were inoculated with only *Scutellospora calospora* and *Claroideoglomus etunicatum* based on previous trials completed in our lab that showed that inoculation with these species had the highest colonization efficiency of these hosts (data not shown).

2.2 | Plant growth conditions

2.2.1 | Asparagus

All pots used in this study were sourced from Stuewe and Sons, Tangent, OR, USA. Asparagus, *Asparagus officinalis* cv. Millenium (Johnny's Selected Seeds, Winslow, ME, USA), seedlings were germinated in a 1:1 mixture of Pro-Mix BX (herein: peat moss; Premier Tech Horticulture) potting mix and sand in 160-ml Cone-tainers™ on February 27, 2020, in a reach-in growth chamber set to 12 h days, 25°C day/15°C night. After 3 months of growth, on May 3, 2020, seedlings were transplanted to 650-ml Deepots™ containing the same media composition plus AMF inoculum (500 spores per pot) or intentionally kept uninoculated. AMF species treatments included *C. etunicatum*, *F. mosseae*, *R. irregularis* (ON205B and AGTIV), *R. intraradices*, *S. calospora*, and a mixed species inocula containing equal volume mixture of all the above species, except AGTIV, plus *C. claroideum*, *G. margarita*, *G. gigantea*, and *S. constrictum* (Table 2). Plants were grown for an additional 14 months in the greenhouse before repotting (June 30, 2021) into 5-L Treepots™ filled with three different media compositions: equal volume mixtures of sand combined with either peat moss (no ERGO), plant matter organic compost sourced from the Rodale Institute (~1.2 mg kg^{-1} ERGO), or freshly collected spent mushroom compost (Mother Earth Organic Mushrooms, West Grove, PA, USA (~5.6 mg kg^{-1} ERGO). Roots were sampled for mycorrhizal colonization analysis at the time of the final transplanting. Shoots were collected and frozen (−20°C) for ERGO analysis on August 18, 2021.

2.2.2 | Black beans

To examine the role of mycorrhizal fungi in ERGO uptake by black beans (*Phaseolus vulgaris* black turtle beans), the plants were grown in

TABLE 2 Host crops, mycorrhizal treatments, and potting media used in each of the trials. Numbers describe the relative volume of each component used in each potting media. AGTIV is the name of a commercial product produced by Premier Tech Horticulture (Riviere-du-Loup, Quebec, Canada) containing the arbuscular mycorrhizal fungus species *Rhizophagus irregularis*.

Host crop	Mycorrhizal treatment	Potting media
Asparagus	Uninoculated Mixed species *Claroideoglomus etunicatum* *Funneliformis mosseae* *Rhizophagus irregularis* *Rhizophagus intraradices* AGTIV (*Rhizophagus irregularis*) *Scutellospora calospora*	1:1 sand: peat moss and 1:1 plant compost: peat moss and 1:1 mushroom compost: peat moss
Black bean	Mock-inoculated Natural community *Claroideoglomus etunicatum* *Claroideoglomus claroideum* *Funneliformis mosseae* *Gigaspora rosea* *Rhizophagus irregularis* *Rhizophagus intraradices* *Scutellospora calospora* *Septoglomus constrictum*	2:2:1 sterilized field soil:peat moss:turface
Wheat and oat	Mock-inoculated *Scutellospora calospora* *Claroideoglomus etunicatum*	2:2:1 sterilized field soil:peat moss:turface and 2:2:1 not sterilized field soil:peat moss:turface

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2-L Treepots™ in a 2:2:1 mix of sterilized field soil:peat moss:turface. AMF inoculum (2000 spores per pot) of *S. constrictum*, *F. mosseae*, *R. irregularis*, *C. claroideum*, *C. etunicatum*, *S. calospora*, *G. rosea*, *R. intraradices*, and the natural community produced from organically managed field soil from the Rodale Institute or an equal volume of "mock inoculum" were incorporated into the media prior to filling the pots (Table 2). In each pot, four black bean seeds were planted on June 3, 2021, and grown outdoors on raised benches. At harvest (August 31, 2021), within each AMF treatment, the seeds from all plants in three pots were composited for ERGO analysis (12 plants per composite sample, 4 replications per treatment). Similarly, composite root samples were collected immediately following harvest and subsampled for staining and determination of AMF colonization. Pods were dried at 55°C for 1 week, then bean seeds were threshed and ground to powder in a tube mill (IKA Tube-Mill 1000, Wilmington, NC, USA) using a speed of 15,000 rpm for 1 min, prior to ERGO analysis.

2.2.3 | Wheat and oats

Wheat and oats were grown in a temperature-controlled greenhouse in 650-ml Deepots using 2:2:1 field soil:peat moss:turface. Field soil was sourced from organically managed agricultural plots at the Rodale Institute and was screened to remove particles >5 mm, then autoclaved at 121°C (probe temperature) for 60 min for sterilized soil treatments or untreated for native soil treatments. AMF inoculum (800 spores per pot) of *S. calospora*, *C. etunicatum*, or an equal volume of "mock inoculum" was incorporated into media prior to filling pots. Seeds of *Triticum aestivum* cv. Surpass or *A. sativa* cv. Corral (Preferred Seed, Buffalo, NY, USA) were pregerminated on moistened paper towels in Petri dishes for 4 days before sowing 1 seedling per pot on May 6, 2021. Three racks of 25 plants were grown for each treatment, and each rack was treated as a replication. Roots were sampled for colonization on June 8, 2021, and the average percent colonization was calculated for each rack. Composite grain samples were collected on August 3, 2021 (wheat) and August 23, 2021 (oats) from the 15 most vigorous plants from each rack, threshed, then prepared for ERGO analysis.

2.3 | Mycorrhizal colonization

To determine the efficiency at which each mycorrhizal species (or mix of species) colonized host roots, we determined percent colonization through staining and the grid-intersect method (Giovannetti & Mosse, 1980). To summarize, roots were soaked at room temperature in 10% potassium hydroxide for ~4 days and brought to a boil to clear and remove pigment from cells. Roots were then acidified in 1% hydrochloric acid for 5 min at room temperature to prepare them for staining in 0.5% trypan blue (Phillips & Hayman, 1970) for 5 min at 90°C.

2.4 | ERGO analyses

ERGO is commonly analyzed using liquid chromatography (LC), and Tsiantas et al. (2021) demonstrated that detection based on mass spectrometry (MS) provides more reliable results than less selective detectors (UV/vis, for example) due to potential chemical matrix interferences. The ERGO concentration of wheat and oats was measured at The Penn State College of Medicine Mass Spectrometry Core Facility, using the LC–MS method reported in Beelman et al. (2021). The ERGO concentration of asparagus and black beans were analyzed at the USDA ARS ERRC using alternative methods described below.

L-(+)-ergothioneine (>98% purity) was obtained from Alfa Aesar (Tewksbury, MA, USA), and L-(+)-ergothioneine-d9 ("highly purified") used as an internal standard (ISTD) for analysis was from US Biological (Swampscott, MA, USA). Optima LC–MS grades methanol and formic acid were from Fisher (Fair Lawn, NJ, USA), and water was obtained from a Barnstead E-Pure system (Dubuque, IA, USA).

Asparagus was analyzed per wet weight, and black beans were analyzed per dry weight (as these are the states that the respective crops are harvested, stored, and/or commonly available to consumers). For asparagus, the frozen tissue samples were weighed and then combined with ~5-ml chunks of dry ice in the bullet-shaped blender container of an IKA Tube-Mill 100 and ground at 5000–8000 rpm for ~1 min until the sample was a homogeneous powder. All samples were transferred to 50-ml polypropylene (PP) tubes and stored at −18°C until analysis.

For both asparagus and black beans, the USDA ARS ERRC laboratory followed a slightly modified LC–MS/MS method described by Sapozhnikova et al. (2014). In brief, 2-g fresh asparagus or 0.5-g dried black bean test portions were weighed into 15-ml PP tubes; 1.5-ml water was added to the dry samples. For spiked test portions, ERGO was added, and in all cases, ERGO-d9 ISTD was added, both in aqueous solutions to yield 1 μg g^{-1} concentrations. Then, 10 ml of 4:1 (v:v) methanol:water was dispensed into the tubes, which were capped, inverted several times, and shaken for 10 min at room temperature using a Glas-Col (Terre Haute, IN, USA) vortex platform shaker at 80% intensity and maximum pulsation. The tubes were then centrifuged for 5 min at 4150 rpm (3711 rcf) at room temperature. For asparagus, 0.4-ml extract (69-mg wet sample equivalent) was pipetted into 0.45-μm PVDF membrane syringeless filter vials and filtered. In the case of black beans, 0.58 ml (25 mg dry sample equivalent) of each extract was transferred to autosampler vials without filtration. Reagent-only and matrix-matched calibration standards of 7 points each of ERGO were prepared in all cases ranging from 0.0625 to 2 μg g^{-1} in wet asparagus and 0.125 to 8 μg g^{-1} in dry black beans. Limits of quantification were <0.05 μg g^{-1} ERGO in the sample analyses, including qualitative identification.

LC–MS/MS was conducted using a Sciex (Foster City, CA, USA) 6500 QTrap coupled with a Shimadzu (Columbia, MD, USA) Nexera II LC. The analytical column was a Phenomenex (Torrance; CA, USA) Luna C8 (100 mm long, 4.6 mm i.d., 3-μm particles) connected to a 5-mm guard column kept at 40°C. An isocratic mobile phase consisting of 70:30 methanol:water containing 0.1% formic acid was used at

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0.5 ml min^{-1} flow rate, leading to a retention time of 2.3 min for both ERGO and ERGO-d9. After each injection, the column was back-flushed for 3 min with 0.1% formic acid in 98:2 methanol:water at 0.15 ml min^{-1} followed by re-equilibration of the mobile phase at 0.5 ml min^{-1} in the forward direction for 4 min. The autosampler tray was maintained at 15°C, and injection volume was 8 µl (4 µl for asparagus).

For detection, electrospray ionization was used in positive mode with 5000 V ion spray and 450°C source temperature. Multiple reaction monitoring was conducted with 75-ms dwell times, entrance potential of 10 V, and unit mass resolution. For ERGO, the precursor ion was *m/z* 230 with declustering potential of 61 V for product ion transitions (*m/z*) of 1) 127, 2) 186, and 3) 60 with collision energy/capillary exit potential (V) of 25/6, 17/8, and 19/10, respectively. For ERGO-d9, the precursor ion was *m/z* 239 with declustering potential of 66 V for product ion transitions (*m/z*) of 127, 195, and 69 with collision energy/capillary exit potential (V) of 27/6, 17/10, and 23/10, respectively. CAD = medium; MR pause = 5 ms; CUR = 25, GS1 = 60; GS2 = 30.

Calibrations were linear using 1/× weighting with $R^2 > .99$. Average recoveries of spiked samples were 74% (14% relative standard deviation, RSD), $n = 9$, in asparagus and 89% (13% RSD), $n = 12$, in black beans. Reference ion ratios measured by peak areas for ERGO were 2/1) 81%, 3/1) 18%, and 3/2) 22%; and for positive identifications in the samples, at least one of the three ion ratios had to fall within ±30% relative difference from the reference ion ratio with all three ERGO ion peaks co-eluting between 2.2 and 2.4 min.

2.5 | Statistical analysis

All statistical analysis was completed in R version 4.2.1. After confirming data were normally distributed via the Shapiro–Wilk test and not heteroscedastic via the Breush–Pagan test, we performed one-way analysis of variance (ANOVA) on plant tissue ERGO concentration for each plant species with AMF treatment as the main factor. After determining the significance of ANOVA ($p < .05$), we performed Tukey–Kramer Honestly Significant Difference (HSD) tests to examine for significant differences between means at the $p < .05$ level. If data did not meet the assumptions of ANOVA (non-normal, heteroscedastic), we used the non-parametric Kruskal–Wallace *H* test followed by Conover's test of multiple comparisons. We performed Pearson's product moment tests to determine significance in correlation between % colonization and plant ERGO concentration for all host plants.

3 | RESULTS

3.1 | Asparagus

Percent root colonization of asparagus varied across mycorrhizal treatments. For asparagus grown in peat moss, the AMF mixed species treatment had the highest level of root colonization (88%), whereas the *C. etunicatum* treatment was the lowest (32%; Table S1). When grown in sterilized plant-compost, *R. irregularis* had the highest rate of colonization (71%), and *F. mosseae* had the lowest (25%). In mushroom compost, the mixed inoculum had the highest rate of colonization (79%), and *F. mosseae* had the lowest (25%). The uninoculated plants had no mycorrhizal colonization (Table S1).

In the peat moss, the only AMF treatment that had an impact on ERGO level was AGTIV, which increased the ERGO concentration of asparagus stalks by 1.3× (Figure 1). In plant compost media, there was no increase in the ERGO content of asparagus in any mycorrhizal treatment compared with the uninoculated control. However, in the mushroom compost media, plants inoculated with the mixed AMF inoculum had 10× more ERGO (Conover's test $p < .05$), and plants inoculated with *R. irregularis* had 6.6× more ERGO (Conover's test $p = .08$) compared with uninoculated plants (Figure 1).

3.2 | Black beans

The level of root colonization of black bean roots varied across mycorrhizal treatments with *S. calospora* being the best colonizer (57% of root length colonized) and *R. intraradices* having the lowest colonization level (13%; Table S2). Bean seeds from plants inoculated with AMF had higher ERGO content than those grown with sterile inoculum for 7 out of the 9 mycorrhizal treatments and displayed the largest increases in ERGO across all crops tested. These increases in ERGO ranged from 72× higher than the level in mock-inoculated plants when grown with the AMF species *S. constrictum* to 36× higher in plants grown with *C. claroideum* (Figure 2).

3.3 | Wheat and oats

In the sterile soil trial, wheat inoculated with *S. calospora* were 42% colonized, and wheat inoculated with *C. etunicatum* were 13% colonized (Table S3) after 30 days of growth. Wheat grown in native soil were 33% colonized, and wheat grown in native soil supplemented by *S. calospora* and *C. etunicatum* were 57% and 30% colonized, respectively. For oats grown in sterile soil, oats inoculated with *S. calospora* were 42% colonized, and oats inoculated with *C. etunicatum* were 5% colonized (Table S3). Oats grown in native soil were 10% colonized, oats grown in native soil supplemented with *S. calospora* were 50% colonized, and oats grown in native soil supplemented with *C. etunicatum* were ~10% colonized (Table S3).

In sterile soil, *S. calospora* increased the ERGO concentration in wheat seeds by 4× and in native soil by 2× relative to mock-inoculated wheat grown in the same media (Figure 3a). *C. etunicatum* did not enhance ERGO uptake in wheat. Similarly, *S. calospora* increased ERGO concentration of oat seeds in sterile soil by 5× and

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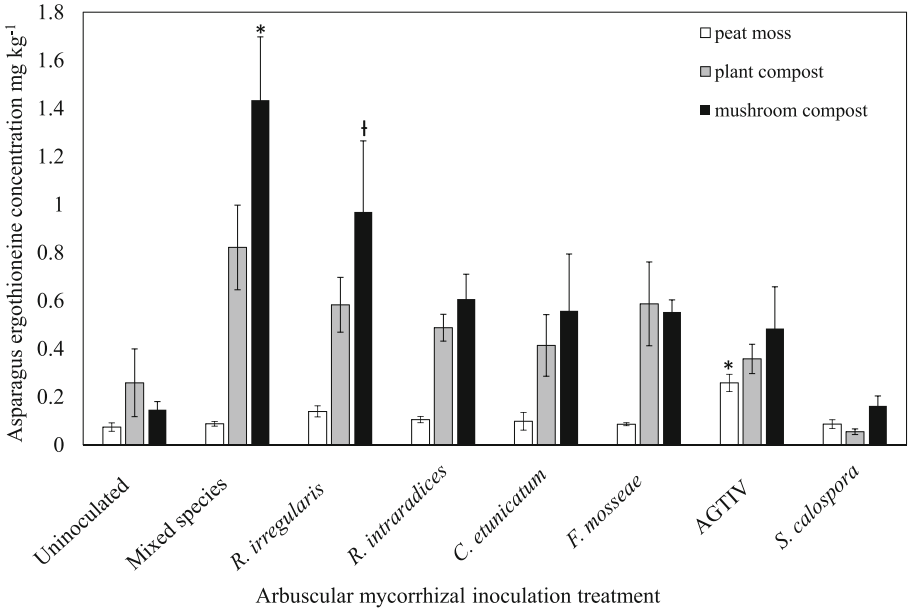


FIGURE 1 Inoculation with a mix of 9 arbuscular mycorrhizal fungus species enhanced the ergothioneine concentration of asparagus tissue when grown in mushroom compost. Values shown are means, and error bars are ±SE. Asterisk indicates significant difference between the arbuscular mycorrhizal treatment and the uninoculated treatment grown in the same media at $p < .05$ and cross indicates significance at $p < .10$. Mushroom compost: Kruskal—Wallis Chi squared = 15.62, df = 7, $p < .05$. Peat moss: Kruskal–Wallis Chi squared = 19.15, df = 7, $p < .05$. Plant and mushroom compost $n = 3$, peat moss $n = 6$. AGTIV is the name of a commercial product containing the arbuscular mycorrhizal fungi species *Rhizophagus irregularis* produced by Premier Tech Horticulture (Riviere-du-Loup, Quebec, Canada).

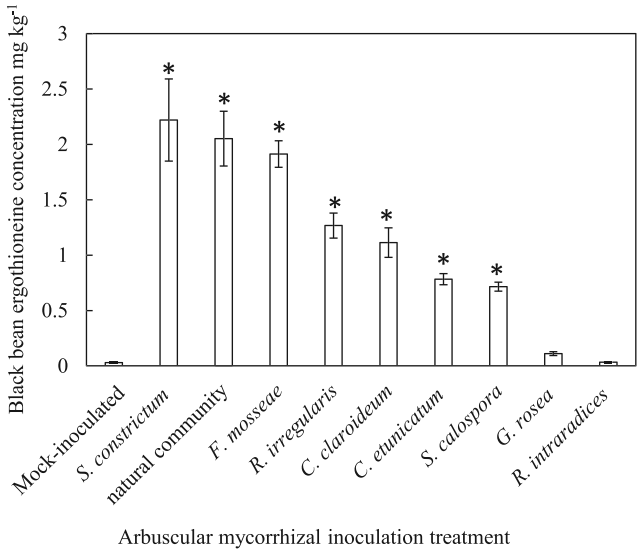


FIGURE 2 Arbuscular mycorrhizal fungus inoculation resulted in increased black bean seed ergothioneine concentration in 7 of the 9 arbuscular mycorrhizal fungus treatments examined. Values shown are means and error bars are ±SE, $n = 4$. Asterisk indicates a significant difference between the arbuscular mycorrhizal fungi treatment and the mock-inoculated treatment (Kruskal–Wallis Chi squared = 36.5, df = 9, $p < .0005$; Conover's test: $p < .05$).

by 2× in native soil compared with mock-inoculated plants grown in the same media, but there was no impact of *C. etunicatum* on ERGO concentration (Figure 3b).

3.4 | Correlation of root colonization and tissue ERGO concentration

There was a positive correlation between % root colonization and ERGO content across all mycorrhizal treatments for asparagus grown in mushroom compost ($p < .05$; Figure 4a) and a marginally significant correlation for asparagus grown in plant compost ($p < .10$; Figure S1) but no relationship for asparagus grown in peat moss. In black beans, across AMF treatments, there was a positive relationship between % colonization and seed ERGO concentration level (Figure 4b). Finally, as in the other crops examined, % colonization was positively correlated with wheat and oat seed ERGO concentration across AMF treatments in both sterile and native soils (Figure 4c–f).

4 | DISCUSSION

ERGO is an antioxidant present in most food crops and has potential as a therapeutic agent, with some advocating that it be considered a vitamin essential for human health (Ames, 2018; Beelman et al., 2020, 2022). However, ERGO is not biosynthesized by animals or plants and the mechanism by which it is taken up from soils is unknown. Here, we show that AMF play a key role in plant tissue ERGO uptake. Whether AMF biosynthesize ERGO and directly transfer it hosts, scavenge ERGO from the soil environment and transfer it to hosts, or they alter plant physiology such that root uptake of ERGO in enhanced, this work shows that plants inoculated with AMF generally

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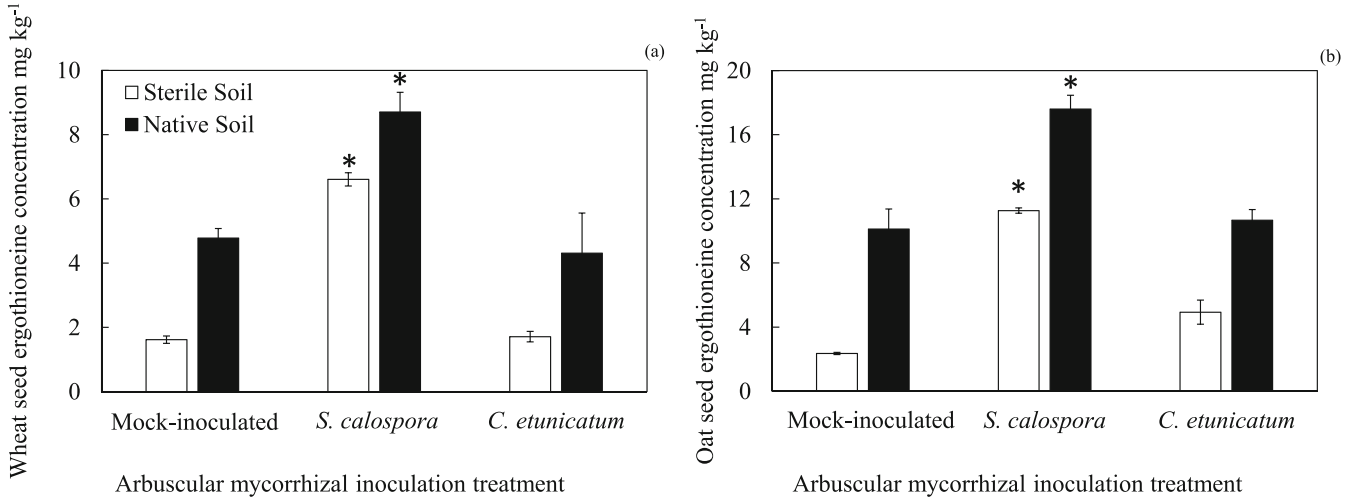


FIGURE 3 *Scutellospora calospora* increased ergothioneine concentration of wheat (a) and oat (b) seeds in both native and sterile soil when compared with the corresponding mock-inoculated and *Claroideoglomus etunicatum* inoculated treatments. Values shown are means, and error bars are ±SE, $n = 9$. Asterisks indicate significant difference between *S. calospora*/*C. etunicatum* treatments and mock within native and sterile soils (wheat analysis of variance [ANOVA]: $F = 14.52$, df = 5, $p < .001$, Tukey Honestly Significant Difference [HSD]: $p < .05$. Oat ANOVA: $F = 34.08$, df = 5, $p < .001$, Tukey HSD: $p < .005$).

have higher ERGO tissue concentration than uninoculated plants. In addition, we found that AMF species display a range of efficiencies at both colonizing host plants and influencing ERGO uptake from the soil to the host.

AMF inoculation of asparagus grown in peat moss, which does not contain ERGO, had a limited effect on tissue ERGO concentration. This suggests that the observed AMF-induced increases in plant ERGO concentration are the result of either AMF transfer of soil ERGO to plants or AMF-induced shifts in plant physiology that enhance root ERGO uptake but are not the result of AMF ERGO synthesis (Figure 1). In fact, ERGO uptake in plants is highest when there is both a belowground ERGO source and AMF to influence host uptake. We make this assertion because asparagus inoculated with mixed AMF species, as well as the single species *R. irregularis*, that was grown in mushroom compost media containing ~5.6-mg ERGO kg^{-1} had higher ERGO content than uninoculated asparagus grown in the same media (Figure 1). Additionally, while wheat and oats grown with the AMF species *S. calospora* had higher ERGO content than plants grown without AMF and with *C. etunicatum*, plants generally had higher ERGO content when grown in native soil than sterile soil (Figure 3). We posit that this may be due to the continuous replenishment of the soil ERGO pool through fungal and bacterial biosynthesis during the duration of the trial, which did not occur in sterile soil.

Across all hosts and mycorrhizal treatments, it appears that AMF colonization generally increased the amount of ERGO in plant tissue (Figure 4). However, our results show that AMF species vary in their efficiency to colonize and enhance ERGO uptake in plant hosts. For example, while the inoculation of black beans with 7 out of 9 AMF treatments enhanced ERGO content by up to 72×, 2 individual AMF species did not enhance ERGO concentration (Figure 2). Differences in the impact of AMF species on nutrient uptake and growth of plant hosts have been documented, and species with a greater ability to access and transfer nutrients to host plants at lower C costs better benefit plant health. (Carrara & Heller, 2022; Cavagnaro et al., 2005; Feddermann et al., 2008; Hart & Reader, 2002; Lanfranco et al., 2018; Munkvold et al., 2004). Past work on mycorrhizal nitrogen (N) uptake and transfer to host plants has shown that direct mycorrhizal uptake of amino acids from soil is an important component of the plant-mycorrhizal N economy (Talbot & Treseder, 2010; Whiteside et al., 2012). Therefore, it is possible that differences in the level of root colonization across AMF species may be driven by differences in the exchange rate of C for nutrients between mycorrhizal species and that ERGO may be traded for plant C as an amino-N source; however, this mechanism requires further testing.

Regardless of the mechanism that explains AMF-induced enhancements in plant ERGO uptake or the role ERGO plays in plant physiology, these results show that AMF act as an important regulator of the transfer of microbially biosynthesized ERGO in soils to plant tissue. AMF inoculation of a variety of crops including eggplant, peppers, potatoes, sweet potatoes, and strawberries has already been shown to enhance yield by up to 15% (Douds et al., 2007, 2008, 2014, 2017; Douds & Reider, 2003), and this study provides evidence that AMF inoculation also improves crop nutrient quality and antioxidant potential. In addition, this work shows that using mixed species inocula is a promising and effective route to enhancing ERGO content in a variety of crops, and some crops, such as black beans, may benefit even more from single species AMF.

Over recent decades, many farmers, activists, and scientists have worked diligently to promote the benefits of conservation agriculture on soil health by encouraging the use of cover crops, crop rotation, and practices that limit soil disturbance

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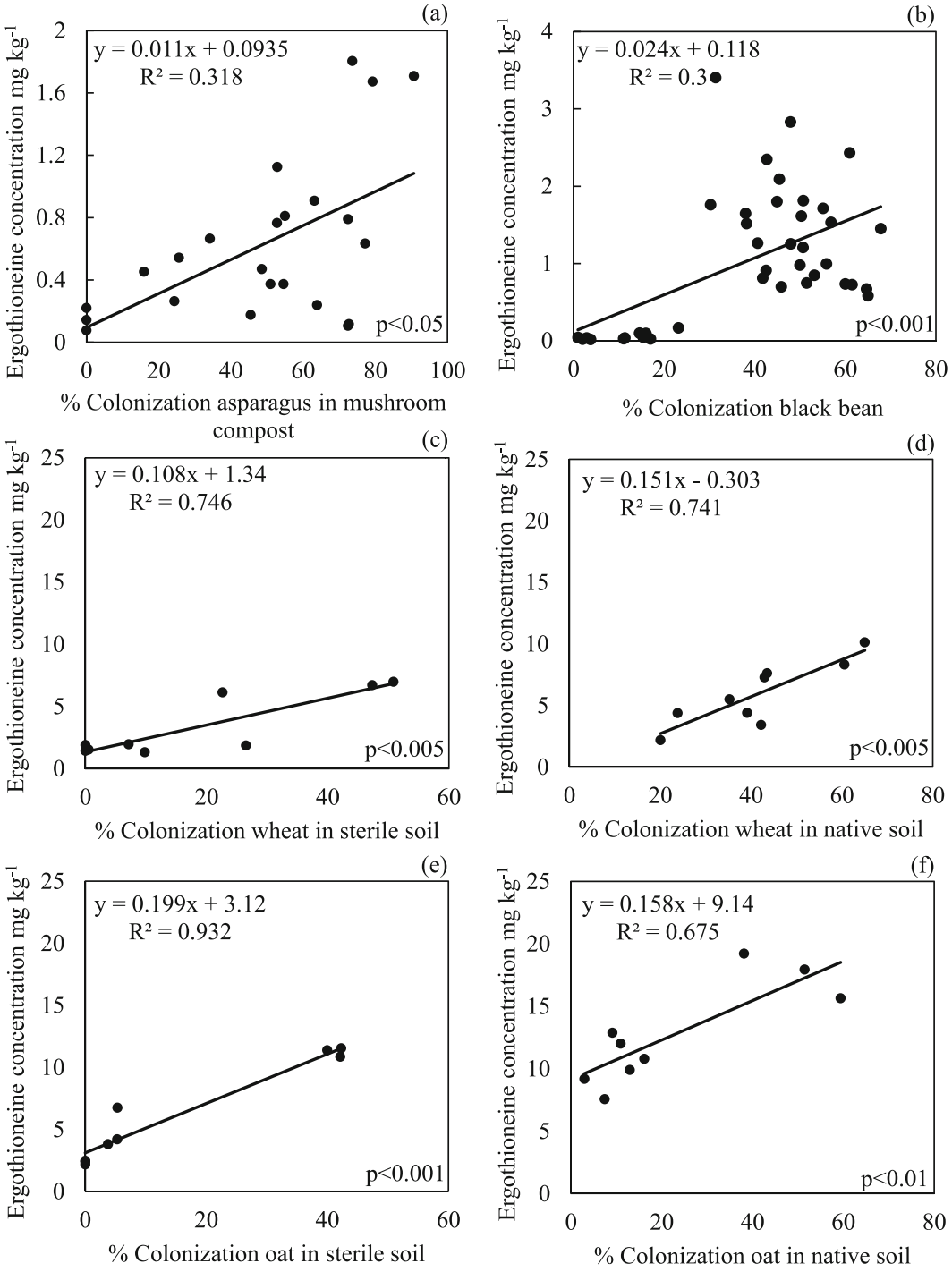


FIGURE 4 Percent mycorrhizal colonization of asparagus, black bean, wheat, and oat all positively correlated with ergothioneine concentration. *P* values are derived from Pearson's product moment correlation test. Each point represents an individual composite plant sample. Asparagus $n = 24$, black bean $n = 40$, wheat $n = 9$, and oat $n = 9$.

(Kassam et al., 2010). While these methods have proven useful in reducing soil erosion, sequestering soil C, and increasing soil microbial populations, direct links to plant nutritional benefits and ultimately human health have been more difficult to prove (Brevik et al., 2020). This work demonstrated that farming practices that bolster the population of AMF and other ERGO producing microbes in cropping systems will increase the ERGO content of a variety of crops. Future research on the effectiveness of AMF inoculation on field crops will further bridge the suspected relationship between soil health and human health.

AUTHOR CONTRIBUTIONS

Wade P. Heller designed the experiments. Wade P. Heller, Joseph E. Carrara, Steven J. Lehotay, Alan R. Lightfield, Dongxiao Sun, John

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P. Richie, and Andrew H. Smith collected, analyzed, and interpreted the data. The manuscript was drafted by Joseph E. Carrara, and all authors read, edited, and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in this article and the supplementary material of this article. Further inquiries can be directed to the corresponding author.

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