Report to the Organic Farming Research Foundation

Conservation of an Endophytic Insect-Pathogenic Fungus for Plant Protection in Organic Cropping Systems

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Project Summary

Farmers and agricultural professionals have a great interest in exploiting beneficial soil organisms, especially in organic systems with their focus on soil health and reliance on natural cycles to manage plant health and pests. Endophytes are microorganisms that form non-pathogenic symbioses with plants and can confer benefits including growth promotion and increased tolerance to environmental stresses that are predicted to increase with climate change. Our long-term goal is to understand how to promote and conserve the beneficial endemic soil fungus, ***Metarhizium robertsii***, as an insect pathogen and a beneficial plant endophyte in organic cropping systems. Within the context of a larger, on-going project to optimize reduced-tillage practices for organic cropping systems, we utilized research station and on-farm observations, a research station field experiment, and greenhouse and lab experiments to answer the questions: **1a**) What factors influence the prevalence of ***M. robertsii*** in soil and endophytic ***Metarhizium*** in corn grown from ***Metarhizium***-inoculated and non-inoculated seed in three cropping systems that impose a range of intensity and frequency of soil disturbances? **1b)** What factors influence the prevalence of ***M. robertsii*** on organic and transitioning agronomic crop farms? **2**) What are the potential effects of drought and flooding on establishment of endophytic ***M. robertsii*** in corn and resulting plant performance?, and **3**) What are the combined effects of water stress and insect feeding on the establishment of endophytic ***Metarhizium*** in corn? **Results:** Early-season prevalence of ***M. robertsii*** in soil in the research station experiment was greater in the system utilizing reduced tillage or shallow tillage compared to the system using inversion tillage but by the late season sample, prevalence among systems was not different. In soil from two organic that utilize tillage and one no-till farm in the second year of transition, prevalence was lowest in soil from the no-till farm in transition to organic production and greater and similar in soil from the two organic farms. Cornell Comprehensive Assessments of Soil Health (CASH) scores were high or very high for all three farms and not related to the prevalence of ***M. robertsii***. Soil moisture and electrical conductivity were positively associated with ***M. robertsii***, while salt concentration and Mg were negatively associated with ***M. robertsii***. On-farm and research farm results suggest that moderate intensity and frequency of tillage are not detrimental to the season-long occurrence of ***M. robertsii***. On farms soil sulfur content (ppm) was a key factor negatively influencing ***M. robertsii***, even though S concentrations fell within the normal agronomic range for Pennsylvania soils. Even though we can readily establish ***M. robertsii*** as an endophyte in corn in the greenhouse, we were not able to establish endophytic ***M.***

robertsii in field corn. Therefore, the most likely practical use of isolates of ***M. robertsii*** similar to the one used in our experiments would be as a soil or seed inoculant for producing seedlings and transplants in steamed growth media. In greenhouse experiments, relative prevalence of ***M. robertsii*** in soil and in plants was lower in the Deficit water treatment than in the Adequate and Excess water treatments. As in the field, S concentration was a negative predictor for endophytic colonization. Soil S in the Excess water treatment was significantly greater than in the Deficit and Adequate water treatments and may have contributes to the relatively low prevalence of ***M. robertsii*** in soil and in the Excess water treatments. The negative impacts of water stress were not alleviated by endophytic colonization with ***M. robertsii***. In greenhouse experiments that combined water stress and insect feeding, treatments, frequency and severity of damage was greatest in the Excess water treatment. There was an unexpected significant interaction between ***Metarhizium*** and Water treatments in which the plants treated with ***M. robertsii*** in the Adequate water treatment suffered a greater frequency of damage than in the uninoculated control. It will be critical to understand under what conditions beneficial endophytes can become detrimental plant stressors.

2. Introduction

In organic agronomic cropping systems, where approved pest control materials are generally not economical to use, growers must rely primarily on cultural practices and biological control to manage pests, and it is critical to build and conserve natural enemy communities to help prevent pest outbreaks. With a focus on soil health and reliance on natural processes to manage pests and soil fertility, organic farmers are eager to exploit beneficial soil organisms to improve system productivity and resilience (Jerkins et al. 2016). Fungal ***endophytes*** (***endo***=inside, ***phytes***=plants) inhabit the tissues of most plants in interactions that range from beneficial to detrimental to the host plant (Aly et al. 2011). Beneficial endophytes can confer stress tolerance, promote plant growth, improve drought tolerance, and alter resource allocation. Other benefits include enhanced uptake of minerals and N use efficiency, and protection against plant pathogens and arthropod pests. Insect pathogens are important but often-overlooked natural enemies of insect pests, and entomopathogenic fungi (EPF) are among the most common insect pathogens. Research has shown that in addition to infecting insects, several species of EPF can colonize plants as endophytes (Behie and Bidochka 2014, Sasan and Bidochka 2012). The focus of our research is the endophytic EPF, ***Metarhizium robertsii***. ***Metarhizium*** has a world-wide distribution in agricultural soils and has been developed as a commercial bioinsecticide (Met52® EC, Novozymes®) for use as a soil drench and foliar applied bioinsecticide in protected and open field applications. Despite much research on this commonly-occurring beneficial fungus applied as a bioinsecticide, our understanding of how management in organic systems affect this soil organism is limited.

3. Objectives Statement

Our long-term goal is to understand how to promote soil health and conserve the benefits of soil-dwelling biological control agents and beneficial, plant-growth promoting fungal endophytes in organic production systems. We examined the role of stress from moisture and pests in mediating endophytic colonization of corn (***Zea mays***) by ***M. robertsii.*** We utilized greenhouse, research-station and on-farm field experiments to determine:

1a) factors that influence the in-field prevalence of endophytic ***Metarhizium*** in corn grown from ***Metarhizium***-inoculated and non-inoculated seed in three cropping systems that impose a range of intensity and frequency of soil disturbances;

1b) factors that influence the prevalence of ***Metarhizium*** in cornfields on three organically-managed grain farms;

2) the potential effects of drought and flooding on ***M. robertsii*** in soil and on establishment of endophytic ***M. robertsii*** in corn and resulting plant performance;

3) the combined effects of water stress and insect feeding by a corn pest (Black cutworm, **BCW**, ***Agrotis ipsilon***) on the establishment of endophytic ***Metarhizium*** and resulting plant performance.

4. Materials and Methods

Objective 1a) Determine factors that influence the in-field prevalence of endophytic ***Metarhizium*** in corn grown from ***Metarhizium***-inoculated and non-inoculated seed in three cropping systems that impose a range of intensity and frequency of soil disturbances

The on-going ***Organic Reduced-tillag***e project experiment (**ROSE**) is a randomized complete block experiment with four replications of four organic systems in a three-year rotation comprised of corn, soybeans, and a small grain. Each complete block contains three crop main-plots (110 x 18 m) and four cropping systems subplots (55 x 9 m). The ***Standard-Till (S1)*** system uses inversion and non-inversion tillage typical of local organic grain farms. The ***Shallow-Till (S2)*** system uses non-inversion tillage with a high-speed disk, an emerging practice. The ***Reduced-Till (S3)*** system integrates no-till planting for soybean and winter spelt. Each system includes a cover crop between cash crops. The ***Perennial (S4)*** system consists of a 3-yr alfalfa (***Medicago sativa***)-orchardgrass (***Dactylis glomerata***) forage that follows spelt. The long-term plan is to manage the experiment as a 6-yr rotation (3-yr alfalfa-grass – corn – soybean – spelt). Over the 3-year rotation, **S4** has the lowest average expected number of soil disturbances (n = 5), **S1** has the most (n = 20), followed by **S2** (n = 15) and **S3** (n = 11).

We collected soil samples (20, 6 in x 2.5 in cores) from each treatments plot in the ROSE to determine relative prevalence of ***M. robertsii*** and soil fertility analysis three times in each growing season (pre-cover crop termination; early crop growth, late season). Relative prevalence in soil was determined by adding 15 larvae of ***Galleria mellonella*** to 500 ml soil in a lidded container. After 10 days, insects were checked for signs and symptoms of infection by ***M. robertsii*** (**Figure 1**). We confirmed the identity of ***M. robertsii*** by morphological and molecular characteristics (Bischoff et al. 2009, Kepler et al. 2015). We stored conidia produced and harvested from single conidium isolates of ***M. robertsii*** on beads (Pro-Lab Diagnostics

Microbank™ Bacterial and Fungal Preservation System) at -80°C for use in the experiments described herein. We submitted the translation elongation factor 1-alpha (TEF1-alpha) sequence of ***M. robertsii*** to NCBI GenBank under accession number MK988559 and the single spore isolated culture to The Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (ARSEF) under the accession number 14325.

We planted ***M. robertsii***-inoculated and non-inoculated seeds in subplots of S1-S3 corn plots and sampled and assayed soil, and plant root and leaf tissues to determine prevalence of endophytic ***M. robertsii***. To treat corn seed, we soaked seed in a spore suspension for 2 h and soak control seeds in water (Ahmad et al. 2020). We planted air-dried ***M. robertsii***-treated and non-treated seeds in early June ~2.5 cm deep and approx. ~15 cm apart in four 3 m sections of row in each corn plot in S1 – 3. We harvested the plants at V4 and evaluated them for endophytic colonization by culturing surface-sterilized root and leaf tissue sections on agar-based selective medium and used standard molecular methods to identify ***Metarhizium*** species (**Figure 2**).

Figure 1. Assay arena for detection of ***M. robertsii*** in soil (left) and assay insect infected by ***M. robertsii*** (right)

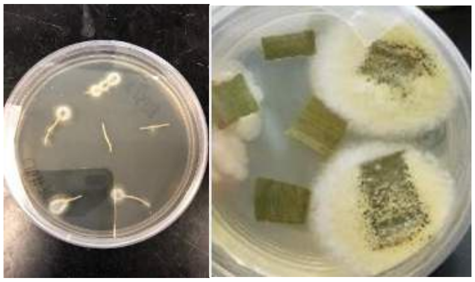


Figure 2. Endophytic ***M. robertsii*** growing from corn root (left) and leaf (right) tissue sections.

Objective 1b) factors that influence the prevalence of ***Metarhizium*** in cornfields on three organically-managed grain farms.

We collected soil samples three times during the growing season from corn fields on three farms (two long-term organic, one in year 2 of transition) to determine soil factors related to the prevalence of naturally-occurring ***M. robertsii.*** Soil was collected in early June when corn plants were just emerging (Early), mid-July when vegetative corn plants about knee high (Mid), and in early November when corn was being harvested or was completely dry and ready for harvest (Late). Soil samples consisted of 20, 6in. x 2.5 in diameter soil cores collected randomly in the field. To determine prevalence in soil, we used the same soil assays as described above for **Obj.**

1a. Portions of each soil sample were also submitted for analysis to the Agriculture Analytical Soil Lab (AASL, **Table 1**) at Penn State for conventional soil fertility analysis and to the Cornell Soil Health Test lab (CASH, **Table 2**) for their Comprehensive Assessment of Soil Health (CASH) testing and scoring to help identify soil factors related to prevalence of ***M. robertsii***.

Farm 1. DJ Farm. The DJ Farm, located near Danville, PA in Montour County, is a mainly conventional grain farm that has been under no-till management since 1970. The farm is currently transitioning some fields to organic using reduced tillage practices. Their transition rotation is comprised of corn, soybeans, small grains, and winter cover crops terminated with a roller-crimper in the spring. DJ Farm is associated with a large poultry producer and uses their grain for poultry feed for their own use and for commercial sales. The sampled field was in the final year of transition from long-term conventional no-till. Corn (Pioneer P0506, untreated) was no-till planted in late May into a standing fall-planted cover crop mixture (cereal rye, crimson clover, balansa clover, winter pea and oats (winter-killed)) and immediately rolled with a roller crimper after planting. This management resulted in a thick and season-long mat of living (first and last sample dates, legume) and dead (for all sample dates, cereal rye) plant material (**Fig. 3, Tables 1, 2**). Because the long-term use of manure has resulted in a buildup of excessive P in the field, soil fertility was managed at planting by an application of 10 lb/ac Chilean nitrate and 3 gal/ac fish emulsion in the row.

Figure 3. Field conditions at Farm 1. DJ Farm on 3 June (left), 12 July (center), and 9 November 2021(right).

Farm 2. DM Farm. The DM Farm, established as an organic farm in 2011, is located near Halifax, PA in Dauphin Co. They produce all-natural beef and organic crops for retail and wholesale. Their crop rotation is comprised of grain and hay crops. The usual crops in the rotation include corn, soybeans, cereal rye, triticale, barley, and alfalfa hay. Corn (Seed Consultants, variety not provided) was planted in the last week of May following a cereal rye cover crop at a planting rate of 29,900/acre into soil prepared with inversion tillage (**Fig. 4, Tables 1, 2**). Fertility was supplied by the application of 3T/ac poultry manure.

The soil was bare and soil moisture good on the first sample date. On the second sample date, corn was at the V9-12 growth stage and the soil was very wet due to the greater than average rainfall. On the final sample date, corn was ready to harvest and very weedy (mostly foxtail, ***Setaria*** species) due to the inability of the farmer to access fields due to persistent wet conditions through the growing season.

Figure 4. Field conditions at Farm 2: DM Farm on 7 June (left) and 11 November 2021 (right).

Farm 3. AZ Farm. The AZ Farm, certified in 2001, is located near Milton, PA in Northumberland County. Their typical six-year rotation is comprised of three years in hay and three years in grain. The usual crops in the rotation include corn, soybeans and winter grains, along with triticale, which serves a dual purpose as chicken feed and cover crop. Other crops include black seed sunflowers, canola and alfalfa/grass hay, and most recently an experimental planting of hemp. AZ Farm uses their grain for organic livestock feed for their own use and for commercial sales. They also direct-market grass-fed beef and poultry. The sampled field was planted on June 1 with a 95-day corn variety (Albert Lea, variety unspecified) at a planting rate of 26,000/acre following a cover crop biculture of 75% triticale:35% Austrian Winter Pea which was terminated by inversion tillage (**Fig. 5, Tables 1, 2**). Fertility was supplied by application of 3 T/ac poultry manure. The soil was bare and soil moisture good for the first two sample dates. Corn had been harvested prior to the final sample date and the soil was covered by corn stover and small winter annual weeds (mainly ***Stellaria media*** and a few brassicas).

Figure 5. Field conditions at Farm3:AZ Farm on 16 June (left), 13 July (center), and 9 November 2021 (right). The field was planted on June 1 with a 95-day corn variety (Albert Lea, variety not specified) at a planting rate of 26,000/acre following a cover crop biculture of 75% triticale:35% Austrian Winter Pea which was terminated by inversion tillage.

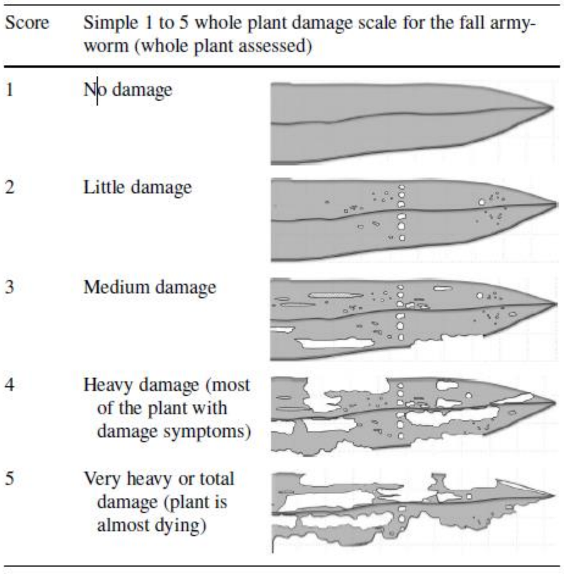
Objective 2) Determine the potential effects of drought and flooding on ***M. robertsii*** in soil and on establishment of endophytic ***M. robertsii*** in corn and resulting plant performance.

To address Objective 2, we conducted a greenhouse experiment to assess the effect of water stress on the recruitment of endophytes by corn (Adejumo and Orole 2010). Treatments included: 1) Application of ***M. robertsii*** spores to soil, adequate moisture [Trt 1]; 2) Application of water, adequate moisture [Trt 2, control for Trt1]; 3) Application of ***M. robertsii*** spores to soil, moisture deficit [Trt 3]; 4) Application of water, moisture deficit [Trt 4, control for Trt 3]; 5) Application of ***M. robertsii*** spores to soil, moisture excess [Trt 5]; 6) Application of water, moisture excess [Trt 6, control Trt 5]. We prepared 10 pots of each treatment and repeated the experiment three times.

We prepared plant growth medium (1 part field soil:1 part organic growth medium by vol., steamed) and planted one untreated organic corn seed per pot and allowed them to grow without water stress until V2. We then initiated deficit water stress by ceasing to water and water saturation by watering excessively. Two days after initiation of the water stress treatments, we inoculated the soil with ***M. robertsii*** spores in water to ***Metarhizium*** treatments, and the same volume of water to control treatments (Ahmad et al. 2020). After 7-10 d of continued water stress, we measured corn height, chlorophyll content (SPAD-502 Plus Chlorophyll Meter, Konica Minolta, Japan), and leaf temperature as indicators of plant performance; and percent plants and percent leaf and root sections colonized by ***M. robertsii*** by standard protocols (Ahmad et al. 2020, Dastogeer 2018). At the end of the experiment, we also measured the relative water content (RWC) of the newest corn leaf as an indicator of the effect of Water treatment (Lugojan and Ciulca 2011). To determine if viable spores of ***M. robertsii*** had been and were still present in the soil among the Water treatments at the end of the experiment, we conducted assays of the soil with sentinel ***G. mellonella***. This sentinel insect assay is an indicator of relative quantity of infective ***M. robertsii*** spores in the soil with greater proportion of infected insects indicating a relatively greater number of spores compared to assays where fewer insects are infected (Zimmerman 1986).

Objective 3) Determine the combined effects of water stress and insect feeding by a corn pest (Black cutworm, **BCW**, ***Agrotis ipsilon***) on the establishment of endophytic ***Metarhizium*** and resulting plant performance.

Because plants experience multiple stresses simultaneously, we conducted a greenhouse experiment to test the interaction of moisture stress and feeding by BCW on the establishment of endophytic ***M. robertsii*** in corn, and on corn performance indicators. Treatments included Trts 1-6 described under Obj. 2, with the application of one 2nd instar BCW to the plants 2 days after soil inoculation with ***M. robertsii*** and to uninoculated control plants. We prepared 10 pots for each treatment and control and repeated the experiment three times. We transferred pre-weighed 2nd instar BCW (Benzon Research Inc.) to plants individually and allowed them to feed until the end of the experiment (~96 hrs). At the end of the experiment, we harvested the newest leaf and primary root tissues to evaluate the endophytic colonization by ***M. robertsii***. As an indicator of potential damage to corn by BCW, we assessed frequency (number) of damaged plants and used a damage rating scale for foliage that ranged from 1 (no damage) to 5 (extensive damage) to measure severity of damage (**Fig. 6,** Toepfer et al. 2021).



Score	Simple 1 to 5 whole plant damage scale for the fall army-worm (whole plant assessed)
1	No damage
2	Little damage
3	Medium damage
4	Heavy damage (most of the plant with damage symptoms)
5	Very heavy or total damage (plant is almost dying)

Fig. 6. Damage scale to rate severity of damage to corn plants by black cutworm (Toepfer et al. 2021).

Changes to methodology in Objective 3: We were unable to recover most of the BCW larvae to determine the effects of water and ***Metarhizium*** treatments on BCW relative growth rate.

Therefore, we used a damage rating scale (Toepfer et al. 2021) as an indicator of larval performance in the Water and ***Metarhizium*** treatments.

Statistical analyses

We performed all statistical analyses in JMP® Pro 16.0.0 (SAS Institute Inc., Cary, NC). We used mixed model ANOVA to determine the effects of treatments on percentage of plants colonized by ***M. robertsii***, plant height, and soil and plant nutrient content. We designated all treatment variables as fixed factors and block (trial replicate number) as a random factor. When the model was significant, we used Tukey's honest significant difference ***post-hoc*** test of means. We considered results of analyses significant at P < 0.05. We used simple regression to determine relationships between single factors, and multiple regression to explain the relationship of environmental factors, to prevalence of ***M. robertsii*** in soil and percentage of endophytically colonized corn plants. Multiple regression models were chosen by minimizing AIC. For all analyses, we transformed proportions using square root arcsine transformation to meet assumptions of normality and equality of variances and to reduce heterogeneity of variances. Data presented in figures and tables are not transformed.

5. Project Results

Obj. 1a) Factors that influence the in-field prevalence of endophytic ***Metarhizium*** in corn grown from ***Metarhizium***-inoculated and non-inoculated seed in three cropping systems that impose a range of intensity and frequency of soil disturbances

Naturally-occurring M. robertsii ***in ROSE***

In 2021, the first year of full implementation of the current iteration of ROSE, sample date had the greatest effect on the prevalence of naturally-occurring ***M. robertsii*** **(**F=13.8, P<0.0001) (**Figure 7**). Prevalence in the Spring sample (pre-cover crop-termination) was significantly greater (P=0.0027, (mean prevalence ± st. error) 22.22±1.62%) than in the Summer (5.21±2.1%) and prevalence in the Fall sample was significantly greater (P<0.0001, 27.86±2.46%) than in the Summer sample. Approach to soil management significantly affected the prevalence of ***M. robertsii*** in the soil (F=2.9, P=0.0390). Prevalence in System 3 (Reduced tillage, 22.42±2.33%) was significantly greater (P=0.0207) than in System 1 (Inversion tillage, 15.7±2.33%) but not different from System 2 (Shallow tillage, 20.82±2.33%) or System 4 (Perennial, 18.57±2.33%). By the Fall sample, prevalence among systems was not different. The interaction between sample date and System was not significant.

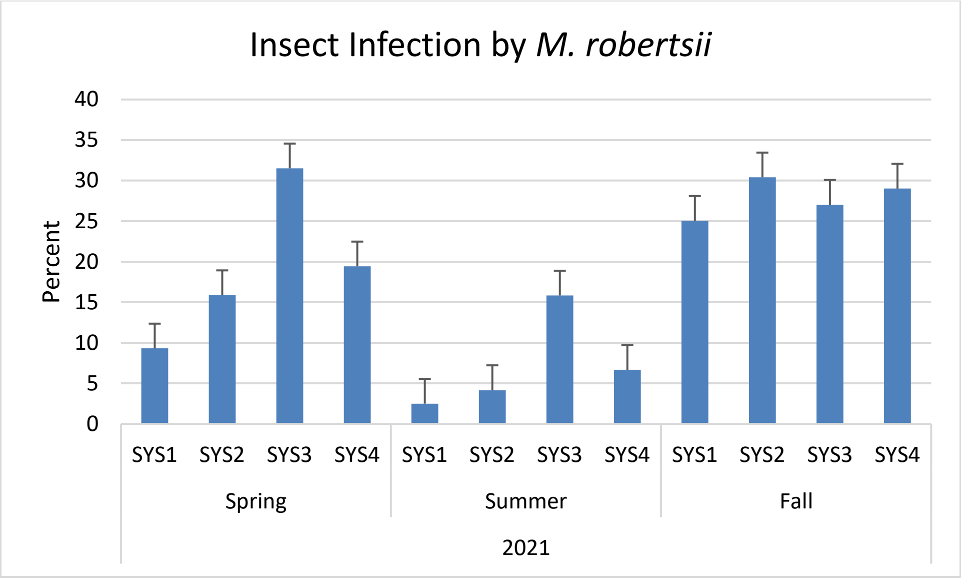


Figure 7. Prevalence of naturally-occurring ***M. robertsii*** in soil in the field experiment to address Objective 1b was conducted in 2021. Prevalence was determined by bioassays of soil with sentinel ***Galleria mellonella*** larvae and is expressed as mean percentage of larvae infected per sample. Key to experimental systems: System 1 = Standard (inversion tillage), System 2 = Shallow Till (high speed disk), System 3 = Reduced (cover crop termination with a roller-crimper, crops no-till planted), System 4 = No tillage (perennial alfalfa/orchardgrass mix).

In a forward selection multiple regression to identify specific soil and management characteristics associated with the prevalence of naturally occurring ***M. robertsii***, four factors were significant (F=13.15, P<0.0001) and explained 27.7% of the variation in infection of sentinel insects. Soil moisture (P<0.0001) and electrical conductivity (P= 0.0122) were positive predictors, and salt concentration (P=0.0014) and Mg (P=0.0025) were negative predictors. Soil moisture alone explained about 16% of the variation in percentage of increased prevalence (percentage of ***G. mellonella*** infected in soil assays), whereas electrical conductivity (uS/cm) explained about 8% of the variation in percentage of decreased prevalence (**Fig. 8**).

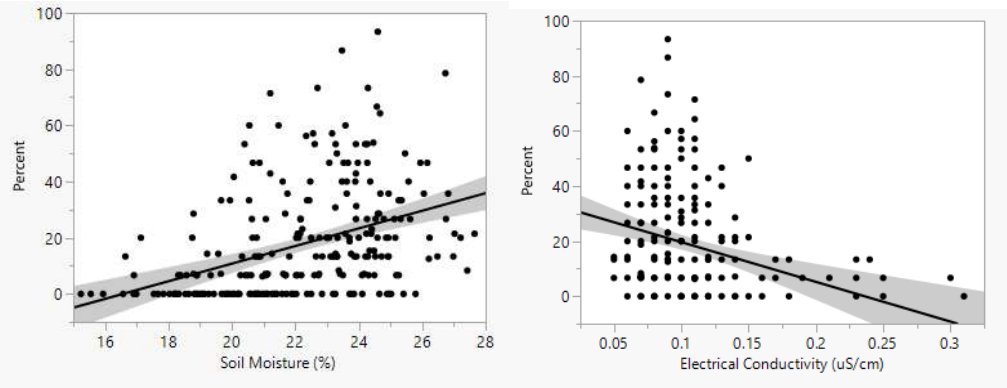


Fig. 8. Two (of four) significant predictors of prevalence of ***M. robertsii*** in soil at the ROSE site.

Effect of seed inoculation on development of endophytic colonization of corn in the field

In the subplot experiment in ROSE to determine if pre-plant treatment of corn seed with ***M. robertsii*** would result in endophytic corn plants, we detected endophytic ***M. robertsii*** in only 1 out of 445 treated plants (2020) and 0 out of 470 (2021) treated plants. To determine why endophytic colonization of corn grown from ***M. robertsii***-inoculated seed in the field was so low, we conducted a replicated experiment in the greenhouse with 8 treatments: Seed treated or untreated and planted in steamed or unsteamed soil, and untreated seed planted in steamed or unsteamed soil inoculated with spores of ***M. robertsii*** or plain water. Plants in the "Seed" treatment were grown from seed inoculated with ***M. robertsii*** (Mr) or left untreated (Control). Plants in the "Soil" treatment were grown from untreated seed but planted in soil that had been inoculated with spores of ***M. robertsii*** (Mr) or with water without spores (Control). The experiment was conducted three times using a total of 324 plants. Data collected included the prevalence of endophytic ***M. robertsii*** in corn plants grown in steamed or unsteamed soil based on the percentage of plants in which we detected ***M. robertsii*** from six leaf and/or six root tissue sections per plant. Results showed that in treatments with seed inoculation and soil inoculation with ***M. robertsii***, endophytic colonization of corn was much lower in unsteamed compared with steamed soil (**Fig. 9**).

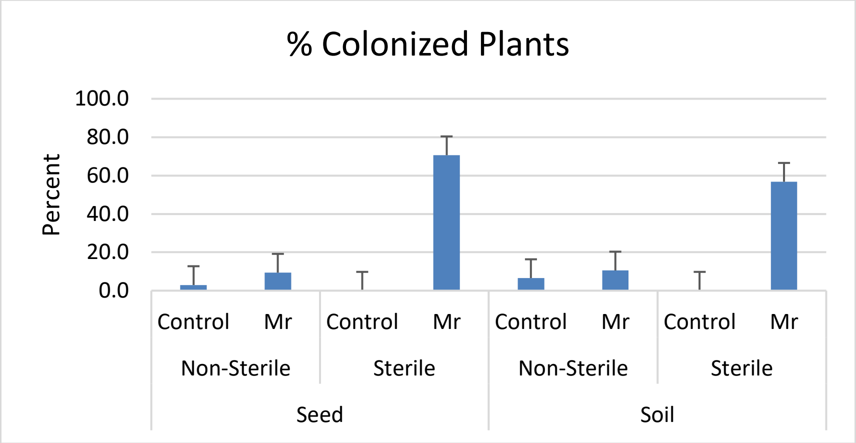


Figure 9. Percentage of corn plants with endophytic ***M. robertsii*** when grown from ***M. robertsii***-treated (Mr) or untreated (Control) seed and planted into steamed (Sterile) or unsteamed (Non-Sterile) soil (left side of graph); and in corn plants grown from untreated seed and planted in ***M. robertii***-inoculated (Mr) or uninoculated (Control) sterile or non-sterile soil (right side of graph).

Obj. 1b) factors that influence the prevalence of ***Metarhizium*** in cornfields on three organically-managed grain farms

On-farm soil factors related to prevalence of M. robertsii

Mean prevalence of ***M. robertsii*** was significantly (P= 0.0495) lower on the farm in transition, Farm 1:DJ Farm (20.0 ± 4.7%), under no-till management, than on long-term organic Farms

2:DM Farm (32.2± 4.7%) and 3:ZA Farm (43.9± 4.7%), which were managed with tillage (**Figure 10).**

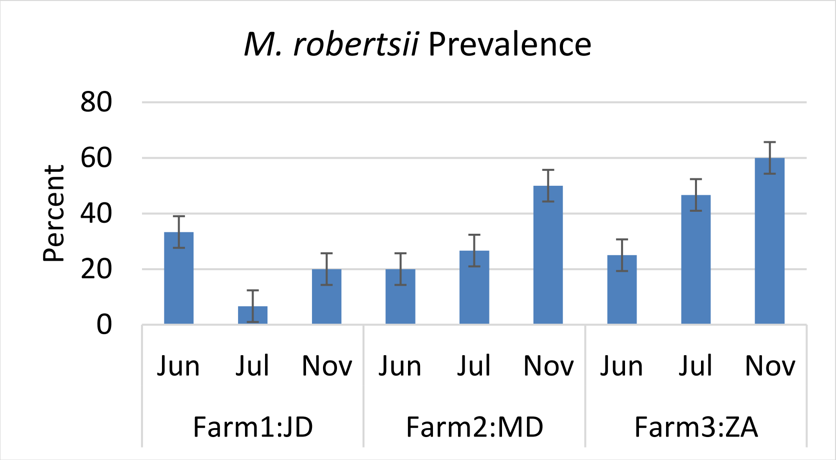


Figure 10. Prevalence of naturally-occurring ***M. robertsii*** in soil on three farms. Prevalence was determined by bioassays of soil with sentinel ***Galleria mellonella*** larvae and is expressed as mean percentage of larvae infected per sample.

On-Farm Cornell CASH Scores

Farm 1 DJ Farm: The overall CASH Quality Score for the June, July, and November samples were Very High, High, and Very High, respectively, but the tests recommended that particular attention be paid to management excessive P to reduce the risk of negative environmental impacts (**Table 2**). The test results were fairly consistent across the growing season, with some of the biological indicators declining over the season.

Farm 2 DM Farm: The overall CASH Quality Score for the June, July, and November samples were High, but the test recommended that particular attention be paid to management of soil biology to optimize soil function (**Table 2**).

Farm 3 AZ Farm: The overall CASH Quality Scores for all dates were High, but the tests recommended that particular attention be paid to management of soil biology to optimize soil function (**Table 2**).

In a forward selection multiple regression that included all CASH measures for each of the three farms separately, no measure was significantly related to the prevalence of ***M. robertsii***. Using CASH measures across all farms, soil clay content, which ranged from 18.4 to 26.5%, was positively associated and explained 64.8% of the variation in prevalence of ***M. robertsii*** (F=15.74, P=0.0054). In a forward selection multiple regression that combined all AASL measures across all farms, soil S (range 9-14 ppm) was the single significant (F=14.6, P = 0.0066) soil factor and was a negative predictor of the prevalence of ***M. robertsii***, explaining 64.8% of variation in prevalence (**Fig. 11**).

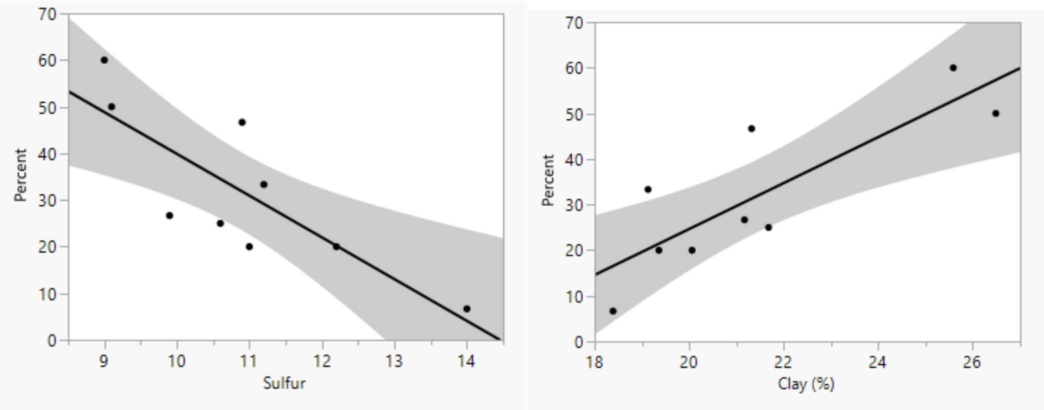


Fig. 11. Significant predictors of on-farm prevalence of ***M. robertsii*** across three farms according to conventional (AASL) soil tests (sulfur) and Cornell CASH test (clay).

AASL Analyses	**DJ Farm**			**DM Farm**			**AZ Farm**		
2021	**Sample Date**			**Sample Date**			**Sample Date**		
Measure	**3 Jun**	**12 Jul**	**9 Nov**	**7 Jun 21**	**19 Jul**	**11 Nov**	**16 Jun**	**13 Jul**	**9 Nov**
Soil class	Silt loam			Loam			Silt loam		
***Metarhizium* (%) (n=15)**	33.3	6.7	20.0	20.0	26.7	50.0	25.0	46.7	60.0
Soil Moisture (%)	21.8	28.0	29.9	16.9	25.8	25.9	22.2	23.9	24.2
Soil organic matter (%)	3.57	3.98	3.61	2.2	2.07	2.09	2.35	2.35	2.35
EC (µS/cm)	121.5	109.9	229.8	302	125.1	156.6	254.3	297.4	102.5
Salts	0.14	0.22	.12	0.25	0.20	.14	0.31	0.3	.09
pH	7.32	7.28	7.29	7.12	7.54	7.34	6.32	6.14	6.48
P (ppm)	478	579	530	64	26	51	66	69	61
K (ppm)	129	149	127	177	79	84	143	115	85
Mg (ppm)	87	97	91	90	98	100	128	114	133
Ca (ppm)	2629.8	3263.8	3195.5	1670.9	1767.9	1670.3	1393.8	1254.8	1390
CEC	14.2	16.2	16.1	9.6	9.9	9.4	10.4	10.3	10.3
%CEC_K	2.3	2.4	2.0	4.7	2.1	2.3	3.5	2.9	2.1
%CEC_Mg	5.1	5.0	4.7	7.8	8.3	8.9	10.3	9.2	10.8
%CEC_Ca	92.6	92.6	93.3	87.4	89.7	8.8	67	60.8	67.6
Zinc (ppm)	12.2	15.8	14.3	2.7	2.1	3.0	3.6	3.6	4.4
Copper (ppm)	2.7	3.3	3.4	2.4	2.4	2.4	1.9	1.9	2.5
Sulfur (ppm)	11.2	14	12.2	11	9.9	9.1	10.6	10.9	9.0

Table 1. Soil fertility analysis (Melich-3 extractant) results from Penn State Agricultural Analytic Soil Lab (AASL). % ***Metarhizium*** is the percentage of waxworm larvae, ***G. mellonella*** (out of 15) that became infected with ***Metarhizium*** in a 10-day assay in which waxworms were placed in a 500-ml sample of soil from the field.

Cornell Soil Health Analyses	**DJ Farm**			**DM Farm**			**AZ Farm**		
2021	**Sample Date**			**Sample Date**			**Sample Date**		
Measure (Rating %)	3 Jun	12 Jul	9 Nov	7 Jun 21	19 Jul	11 Nov	16 Jun	13 Jul	9 Nov
Soil class	Silt loam			Loam			Silt loam		
***Metarhizium* (%) (n=15)**	33.3	6.7	20.0	20.0	26.7	50.0	25.0	46.7	60.0
% Sand	20.1	19.7	22.6	36.2	44.4	30.1	14.1	15.5	12.6
% Silt	60.7	61.9	57.3	44.4	46.4	43.4	64.2	63.1	61.8
% Clay	19.1	18.4	20.1	19.4	21.2	26.5	21.7	21.3	25.6
Predicted water capacity (rating)	.26 (94.3)	.27 (95.5)	0.26 (94.3)	.21 (77.2)	.22 (82.9)	.22 (83)	.27 (95.5)	.27 (96.1)	0.27 (94.0)
Aggregate Stability (rating)	38.2 (65.5)	24.7 (36.2)	17.04 (21.9)	20.6 (28.2)	6.6 (8.7)	5.0 (7.4)	15.4 (19.4)	12.9 (15.8)	3.3 (6.2)
Soil organic matter (rating)	4.0 (87.9)	4.1 (88.2)	4.05 (87.8)	2.6 (32.3)	2.4 (25.2)	2.2 (17.9)	2.7 (36.2)	2.6 (33.8)	2.4 (25.6)
Total organic C	2.4	2.4	2.62	1.4	1.2	1.1	1.4	1.2	1.1
Total N	0.23	0.23	0.23	0.13	0.11	.10	0.14	0.12	0.12
Soil protein index, mg/gm soil (rating)	6.6 (51.7)	5.9 (43.5)	7.9 (67.1)	5.3 (35.7)	5.9 (30.1)	4.9 (31.2)	5.3 (35.8)	4.8 (30.1)	5.4 (36.9)
Soil respiration (rating)	0.68 (61.4)	0.44 (29.5)	0.47 (33.8)	0.50 (37.2)	0.49 (36.3)	0.55 (42.8)	0.39 (24.8)	0.50 (37.2)	0.55 (43.8)
Active C, ppm (rating)	772.6 (92.9)	890.2 (98.3)	929.8 (98.9)	412.3 (31.8)	382.9 (26.3)	432.6 (35.8)	452.7 (39.9)	439.9 (37.3)	498.2 (49.6)
pH (rating)	6.9 (100)	7.1 (100)	7.16 (100)	6.9 (100)	7.1 (100)	7.3 (100)	6.1 (93.8)	6.0 (86.2)	6.6 (100)
P, ppm (rating)	290.1 (100)	373.7 (100)	285.5 (100)	10 (100)	7.4 (100)	3.4 (97)	7.7 (100)	7.9 (100)	11.3 (100)
K, ppm (rating)	160.9 (100)	203.4 (100)	171.1 (100)	180.5 (100)	98.5 (100)	87.7 (100)	174.9 (100)	156 (100)	101.2 (100)
Mg	96.7	95.4	86.4	76	90.4	89.5	125.6	122.8	119.3
Iron	0.9	0.9	0.5	1.4	1.1	0.7	0.8	0.8	0.5
Manganese	5.7	5.8	5.9	4.8	8.4	4.0	6.3	6.3	4.7
Zinc	2.7	2.8	3.5	0.5	0.4	0.4	0.9	0.9	1.0
Minor elements rating	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Overall Score	85.4 Very High	79.1 High	80.4 Very High	64.2 High	62.3 High	61.6 High	64.5 High	63.6 High	65.7 High

Table 2. Cornell Soil Health Test results and soil fertility analysis (Morgan extractant). Key to Cornell Soil Health Test ratings: A rating below 20 indicates ***Very Low*** (constraining) soil functioning and is color-coded red; between 20 and 40 indicates ***Low*** soil functioning and is color-coded orange; between 40 and 60 indicates ***Medium*** soil functioning and is color-coded yellow; between 60 and 80 indicates ***High*** soil functioning and is color-coded light green; and 80 or greater indicates ***Very High*** soil functioning and is color-coded dark green. The Overall Quality Score at the bottom of the table is an average of all ratings and provides an indication of the soil's overall health status. %***Metarhizium*** is the percentage of waxworm larvae, ***G. mellonella*** (out of 15) that became infected with ***Metarhizium*** in a 10-day assay in which waxworms were placed in a 500-ml sample of soil from the field.

Obj. 2) the potential effects of drought and flooding on ***M. robertsii*** in soil and on establishment of endophytic ***M. robertsii*** in corn and resulting plant performance

At the end of the greenhouse experiment, relative abundance of viable ***M. robertsii*** in the soil, as detected by infection of sentinel ***G. mellonella*** in soil assays was affected by Water treatment (F=9.57, P <0.0001). Relative abundance of ***Metarhizium*** spores in the Deficit water treatment was significantly lower than in the Adequate (P< 0.0005) and Excess (P = 0.0009) Water treatments. (**Figure 12**).

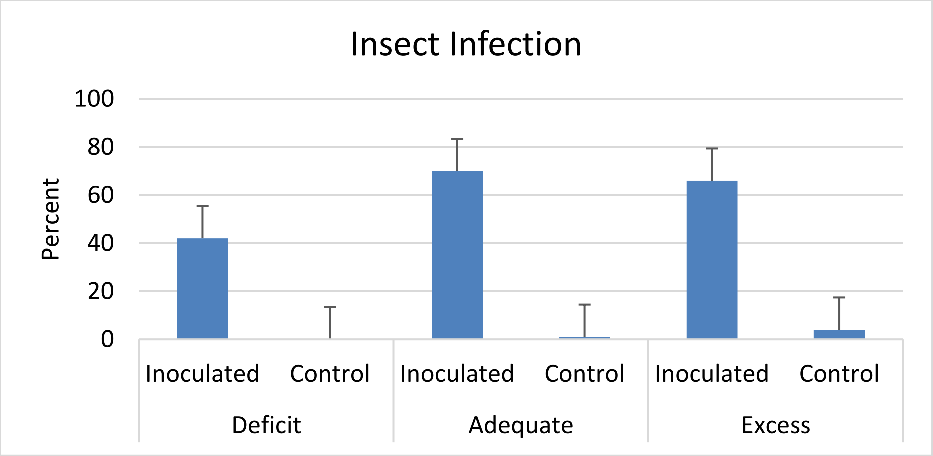


Figure 12. Relative prevalence of ***M. robertsii*** in untreated (Control) soil and in soil that had been inoculated with spores of ***M. robertsii*** (Inoculated), as determined by assays with ***G. mellonella*** larvae, in soil in which corn was grown in the Deficit, Adequate, or Excess water treatments.

At the end of the greenhouse experiment we assayed root and leaf tissue of all plants to determine if water stress treatment affected colonization of corn roots and foliage by ***M. robertsii***. We detected ***M. robertsii*** in leaf tissue of only two plants, so they were excluded from further analysis. As we frequently detect ***M. robertsii*** in the leaf tissue of corn plants grown from inoculated seed (Ahmad et al. 2020), we believe that the low detection rates with inoculation by soil drenching used here were due to insufficient time during the ~10-day exposure period for the establishment of a level of systemic colonization detectable by the culture method used in this experiment. Water treatment and the interaction between Water and ***Metarhizium*** treatment had a significant effect on endophytic colonization (Water treatment F=9.57, P=0.0001; ***Metarhizium*** treatment F=67.3, P<0.0001; Interaction F=5.89, P=0.0034) (**Figure 13**). Colonization of root tissue ***M. robertsii***, in the Deficit Water treatment was lower than in the Adequate Water treatment (P=0.0005) and significantly greater than in the Excess water treatment (P=0. 0009), but colonization in the Adequate and Excess water treatment was not different.

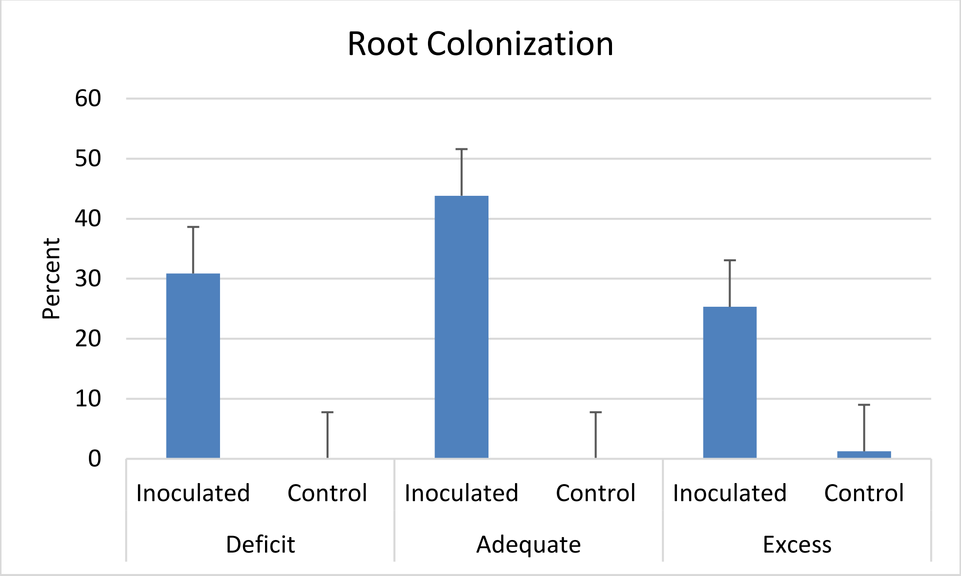


Figure 13. Prevalence of root colonization of ***M. robertsii*** in untreated (Control) soil and in soil that had been inoculated with ***M. robertsii*** (Inoculated) in the Deficit, Adequate, and Excess water treatments.

There was a significant positive relationship ($r^2 = 0.277$, $P<0.0001$) between the percentage of sentinel insects infected by ***M. robertsii*** and the proportion of root tissues colonized by ***M. robertsii***, indicating that a greater the number of viable spores in the soil is associated with a greater probability of colonization of roots (**Figure 14**).

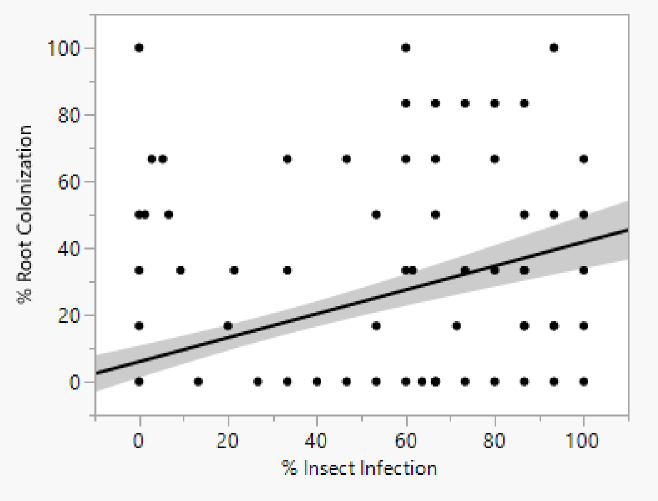


Figure 14. Relationship between prevalence of ***M. robertsii*** in soil (as determined by percentage of sentinel ***Galleria mellonella*** larvae infected with ***M. robertsii*** from soil used in the experiment) and the detection of endophytic ***M. robertsii*** in corn roots (percentage of plants in which we detected endophytic ***M. robertsii*** in roots).

In a multiple regression analysis to determine significant soil factors for root colonization, four factors were significant (r^2=.316, F=19.83, P<0.0001) and explained approximately 32% of variation in endophytic colonization of corn. ***M. robertsii*** prevalence in soil (percentage of infected insects, P = 0.0000), P concentration (P=0.03511), and Mg concentration (P=0.03365) were positive predictors, and S concentration (P=.00249) was a negative predictor for endophytic colonization.

Because of the significance of soil S content as a negative predictor of ***M. robertsii*** prevalence in the assays of field soil under Objective 1, and endophytic colonization was lowest in the Excess water treatment in Objective 2 experiments, we analyzed the differences in soil S at the end of the greenhouse experiments. Water treatment, but not ***Metarhizium*** treatment had a significant (F=21.22, P <0.0001) effect on soil S concentration. Soil S in the Excess water treatment was significantly greater than in the Deficit (P<0.0001) and Adequate (P<0.0001) water treatments (**Fig. 15**).

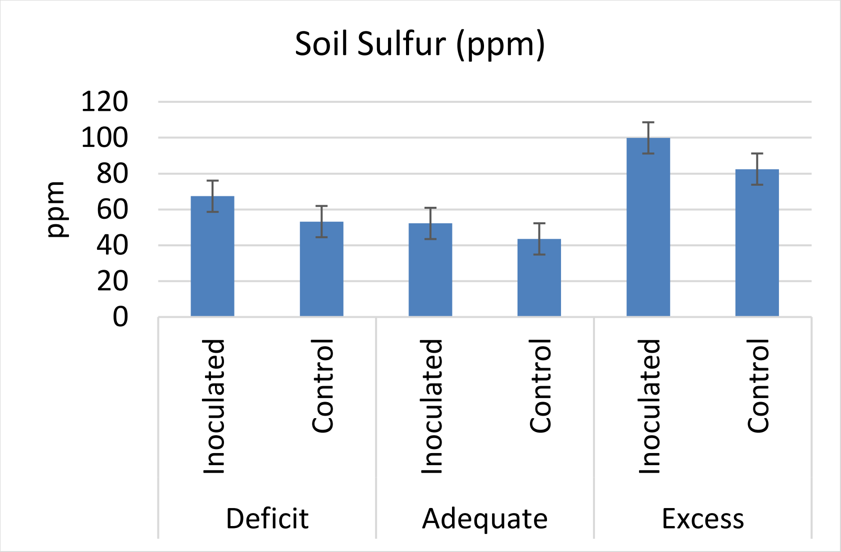


Fig. 15. Soil sulfur concentrations at the end of the greenhouse experiment described under Obj. 2.

To assess plant performance in the ***Metarhizium*** and Water treatments we measured indicators including Relative Water Content (RWC), leaf temperature, plant height, chlorophyll content of corn plants (**Table 3**). Water treatment, but not ***Metarhizium*** treatment, affected the plant performance indicators. Water treatment affected the RWC of corn foliage at the end of the experiment (F = 3.71, P=0.0266; Table 3). The RWC of leaf tissue in the Deficit treatment was significantly lower (P=0.0244) than in the Excess water treatment. Water treatment affected the temperature of corn foliage (F = 6.16, P=0.0027; Table 3). The temperature of corn foliage in the Deficit water treatment was significantly higher (P=0.0020) than in the Adequate water treatment, but not different from the Excess water treatment. Water treatment affected the height of corn plants at the end of the experiment (F = 138.16, P<0.0001; Table 3). The height of corn plants in the Deficit treatments were significantly shorter than plants in the Adequate (P<0.0001) and Excess (P=0183) water treatments. Water treatment affected the chlorophyll content of corn foliage (F = 3.48, P=0.0333; Table 3). The chlorophyll content of corn foliage in the Deficit water treatment was significantly lower (P=0.0248) than in the Adequate water treatment, but not different from the Excess water treatment.

Water Treatment	*M. robertsii* Treatment	Leaf Relative Water Content	Leaf Temperature (F)	Chlorophyll Content	Plant Height (cm)
Deficit	Inoculated	89.7	78.5	41.7	103.1
	Control	81.0	78.5	42.7	99.4
Adequate	Inoculated	93.9	77.5	44.1	123.9
	Control	93.9	77.5	45.6	122.1
Excess	Inoculated	97.2	77.8	42.3	125.9
	Control	97.4	77.9	44.7	129.4

Table 3. Mean Relative Water Content (RWC), leaf temperature, total chlorophyll content (SPAD, and end-of experiment height of corn plant treated or untreated with ***M. robertsii*** and subjected to Deficit, Adequate, or Excess water treatments.

Obj. 3) Determine the combined effects of water stress and insect feeding by a corn pest (Black cutworm, **BCW**, ***Agrotis ipsilon***) on the establishment of endophytic ***Metarhizium*** and resulting plant performance

Because plants experience multiple stresses simultaneously, we tested the interaction of moisture stress, ***M. robertsii*** colonization of corn, on damage to corn plants by BCW larvae. There was no difference in the numbers of corn plants (frequency) damaged by BCW due to ***Metarhizium*** treatment. Water treatment had a significant effect on frequency of damage (F=7.45, P=0.0008) in which frequency of damage in the Excess water treatment was greater than in the Adequate (P=0.0006) water treatment (**Fig. 16**). There was a significant interaction between ***Metarhizium*** and Water treatments (F=9.22, P=0.0001) in which the Inoculated plants in the Adequate water treatment suffered a greater frequency of damage than in the uninoculated control. Frequency of damaged plants in Inoculated and Control treatments was similar in the Deficit and Excess water treatments. Water treatment, but not ***Metarhizium*** treatment, had a significant effect on severity of damage to corn foliage from BCW feeding (F=3.06; P=0.0491). Severity of foliar damage was greater in the Excess compared with the Adequate water treatment (**Fig. 17**; P=0.0420).

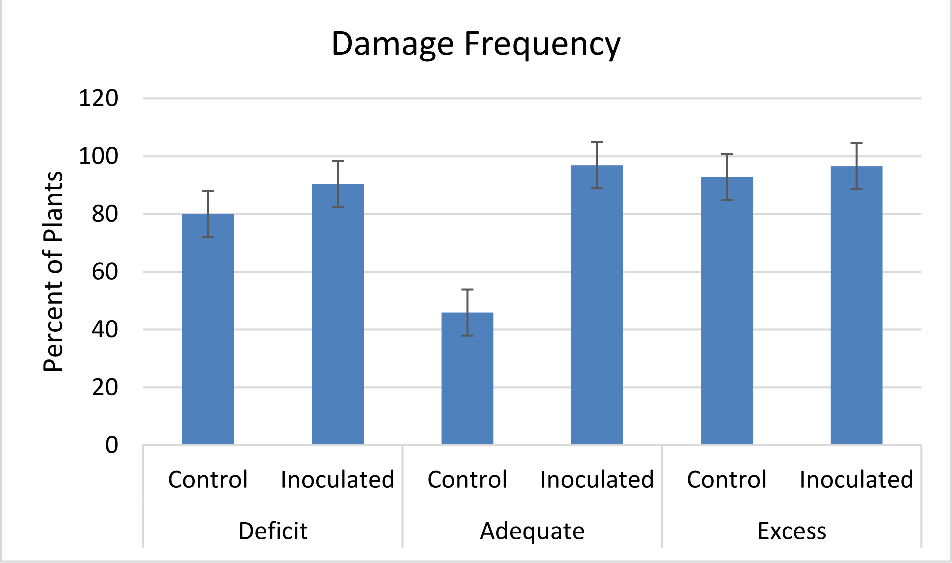


Fig. 16. Percentage of corn plants showing any foliar damage (frequency) in the Deficit, Adequate, and Excess water treatments and uninoculated (Control) or inoculated with ***M. robertsii***.

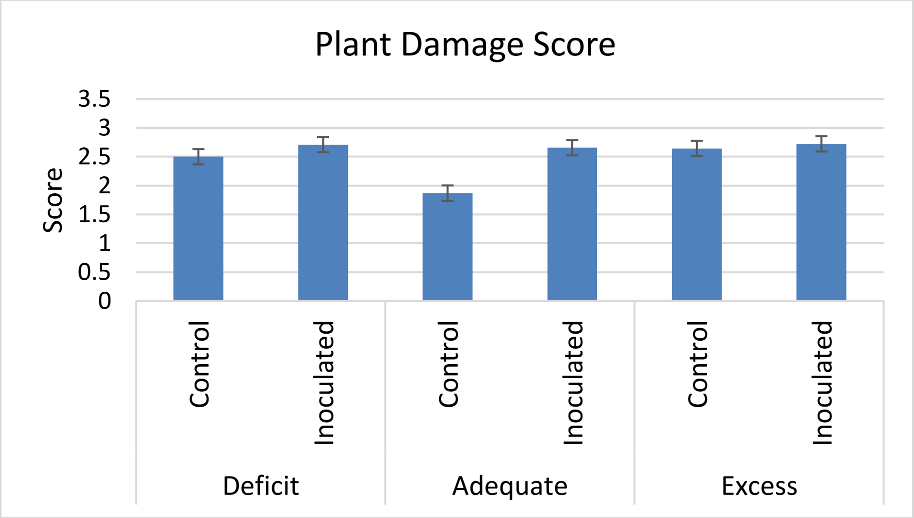


Fig. 17. Severity of foliar damage based on a scale of 1 (no damage) to 5 (severe damage) in corn grown in the Deficit, Adequate, and Excess water treatments and uninoculated (Control) or inoculated with ***M. robertsii***.

In multiple regression analysis to identify plant factors related to the severity of plant damage, four factors were significant (r^2=.5539, F=20.92, P<0.0001) and explained 55.4% of the variation in damage severity. Plant S content (%) (P<0.0001) and soil moisture positive (P<0.0001) were positively related to the severity of plant damage, while plant Mg content (%) (P=0.00029) and plant K (%) (P=0.00041) were negatively related to the severity of plant damage. Water treatment, but not ***Metarhizium*** treatment, significantly (F=3.78, P=0.0280) affected Plant S content, the strongest predictor. Plant S content in the Deficit water treatments was significantly lower than in the Adequate water treatment plants (**Fig. 18**). There was a significant relationship between S content of the soil and S content of plants (r^2=0.2507, F=54.89, P<0.0001) **(Fig. 19)** in which soil S content explained about 25% in the variation of plant S content.

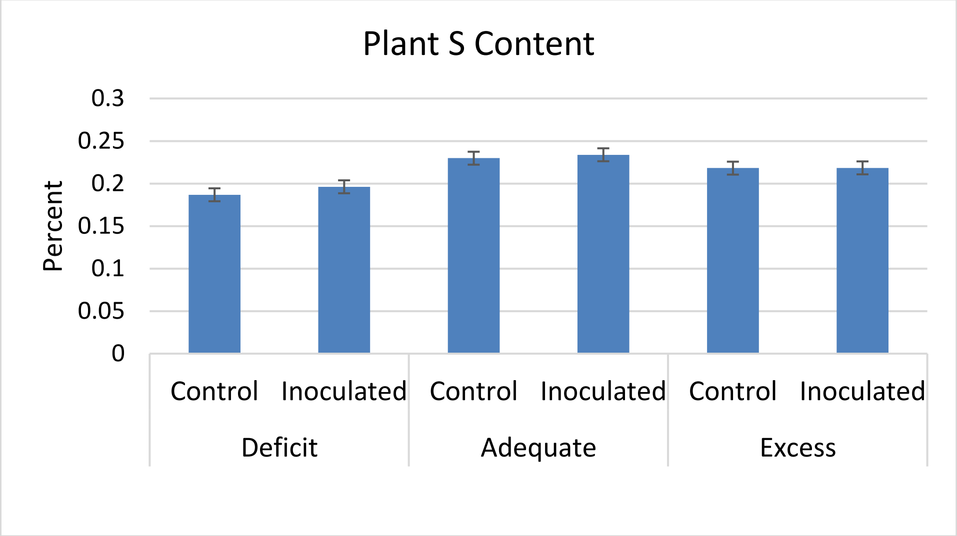


Fig. 18. Plant S content of corn grown in the Deficit, Adequate, and Excess water treatments and uninoculated (Control) or inoculated with ***M. robertsii***.

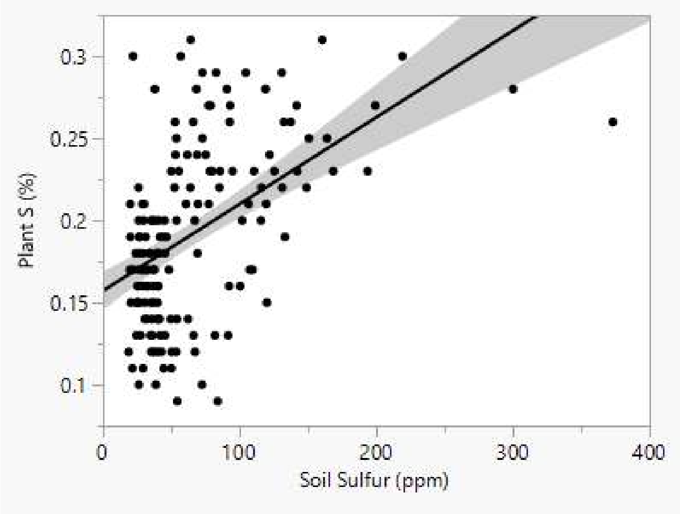


Fig. 19. Relationship between soil S content (ppm) and plant S content (%).

6. Discussion and Conclusions

Obj.1. Previous studies have found that in addition to being a commonly occurring beneficial insect pathogen in soil, endophytic ***Metarhizium*** colonization of plants can positively impact plant performance (Ahmad et al. 2020, reviewed in Vega 2018, Vidal and Jaber 2015). Therefore, it is worthwhile understanding the factors that contribute to the prevalence of ***Metarhizium*** in the field to facilitate its use in conservation biological control of insect pests and potentially as a natural plant growth promotor and protectant. This is especially important for organic grain farms, where the numbers of materials available for rescue pest management are limited and generally not economical to use.

We found that the level of soil disturbance imposed by tillage at the research station and on the two organic farms that utilize tillage did not have a lasting negative effect on the detection of ***M. robertsii***. Even where inversion tillage resulted in decreased detection early in the season, prevalence recovered by the end of the season and was similar among tilled, reduced-till, and no-till systems. These results suggest that naturally-occurring ***M. robertsii*** populations were able to recover from early-season soil disturbance and that, at least for some beneficial microorganisms, some tillage should not be detrimental to populations in the short-term. Our results confirm earlier observations in which the effects of soil disturbance on naturally-occurring ***M. robertsii*** are quadratic (Jabbour and Barbercheck 2009), with low levels of soil disturbance and frequent intensive tillage events resulting in decreased levels of detection, and moderate levels of disturbance associated with greater detection of ***M. robertsii*** in soil. ***Metarhizium*** spp. exist in the soil as infectious spores and can be highly aggregated around infected and sporulating insect cadavers. Increased detection after tillage may be due to breaking up aggregations and mixing them throughout the soil column, increasing the likelihood of detection. Infections of insects by ***M. robertsii*** and growth on organic substrates through the season could also have contributed to the general increase in prevalence that we observed on the two organic farms (Stone and Bidochka 2020).

Many growers are interested in measuring soil health and are curious about tests such as the Cornell Comprehensive Assessment of Soil Health (CASH, https://soilhealth.cals.cornell.edu/). Overall, CASH scores for corn on three farms were high (tilled, organic) or very high (transitioning, no-till). Even though both the conventional soil test and the CASH test include several variables useful for guiding management, only four measures predicted the prevalence of ***M. robertsii***. In our field studies, many of the factors that affected the prevalence of ***M. robertsii*** in soil are manageable. For example, soil moisture and electrical conductivity were positively associated with ***M. robertsii*** and can be managed through controlling disturbance and building soil organic matter. The positive effect of soil moisture may help to explain the generally lower prevalence of ***M. robertsii*** in soil in the relatively drier summer soil samples. Salt concentration (EC, electrical conductivity) and Mg were negatively associated with ***M. robertsii*** and can be managed where appropriate, for example, by applying gypsum or high calcitic lime and by not relying exclusively on high-salt composts or animal manures for soil fertility. In non-saline soils, EC is affected by cropping, irrigation, land use, and application of fertilizer, manure, and compost. Management that leads to low organic matter, poor infiltration, poor drainage, saturated soil, or compaction can increase EC. In general, soil microorganism activity tends to decline as EC increases. This impacts important soil processes such as respiration, residue decomposition, and N cycling in addition to potential impacts on microbial control of pests (Smith and Doran 1996).

Our inability to use ***M. robertsii***-inoculated seed to produce endophytic corn plants in the field was somewhat unexpected. In greenhouse experiments, we have achieved up to 91% of plants developing a systemic colonization when grown from ***M. robertsii***-treated seed, and endophytically colonized plants suppressed the relative growth rate of BCW larvae, and were significantly greater in height and above-ground biomass compared to control plants (Ahmad et al. 2020). The drastically lower rates of endophytic colonization of corn plants by ***M. robertsii*** in the field compared to the greenhouse suggests that ***M. robertsii*** is not a strong competitor with other naturally-occurring rhizosphere or endophytic microbes. Our result of lower endophytic colonization in corn grown in steamed vs unsteamed field soil confirmed similar observations made by Parsa et al. (2018). It was interesting that we did detect a very low level of endophytic colonization of corn plants in the unsteamed soil treatments, indicating that at least a low level of natural infection can occur from field soil. Our results suggest that until more rhizosphere-competitive strains can be isolated, the most likely practical use of ***M. robertsii*** as a plant protectant and growth promotor will be in situations in which soil has low abundance and diversity of potential microbial competitors or antagonists, ***e.g***., in medium for producing seedlings in greenhouses, high tunnels and other protected environments.

In multiple regression analysis of soil factors that were related to the prevalence of ***M. robertsii*** on the sampled farms, clay content and sulfur concentration were significant. Clay content is related to the ability of soil to hold moisture, which is generally positively related to prevalence of ***M. robertsii***. This strong positive relationship between clay content and ***M. robertsii*** also indicates that soil texture, an inherent property that is difficult to change through management, can be a more important property than dynamic properties that can be changed through management. The negative effect of sulfur on ***M. robertsii*** confirms the observations of previous research. Sulfur is fungicidal and ***M. robertsii*** appears to be highly sensitive to soil sulfur concentrations. This is consistent with earlier findings from field experiments, even though sulfur concentrations fell within the normal agronomic range for PA soils (10-25 ppm) (Jabbour et al. 2009, Randhawa et al. 2018). In previous experiments to determine the effects of cover crops on ***M. robertsii***, detection was lowest where brassica cover crops were grown (Randhawa et al. 2018). This could be due to the inhibitory effects of glucosinolates or other sulfur-containing secondary metabolites associated with brassicaceous plants (Steinwender et al. 2015, von Roepenack-Lahaye et al., 2004) and Klingen et al. (2002) reported that ***Metarhizium*** spores did not germinate when exposed to 100 ppm glucosinolates in a laboratory experiment. Practical recommendations that could be implemented for conservation of ***M. robertsii*** in soil would include using practices that can conserve moderate levels of soil moisture, such as mulching and avoiding excessive applications of sulfur-containing materials, such as fungicides, or animal manures that can contain high levels of sulfur.

Obj. 2. Water stress, both deficit and excess, associated with increasing variability in frequency and intensity of rainfall, is predicted to become more severe with climate change (Trenberth 2011). In our greenhouse experiment to determine the impact of water stress, water treatment, specifically water deficit, negatively affected corn plant performance as determined by Relative Water Content (RWC), leaf temperature, chlorophyll content. Treatment of soil with ***M. robertsii*** did not improve or worsen effects of Water treatment.

Consistent with field observations described in Objective 1, results from greenhouse experiments showed that sulfur concentration in soil was negatively associated with ***M. robertsii*** in both the soil and in plants. Soil S was greatest in the Excess water treatment and soil S was positively related to foliar S in our experiments. We suggest that the direct negative effects of excess water result in relatively low oxygen levels, greater potential for microbial degradation of ***M. robertsii*** spores in soil, and high soil and plant sulfur content.   These results support recommendations stemming from Obj. 1 results, and point to the importance of managing soil drainage to avoid waterlogging and build up of soil S to conserve ***M. robertsii***.

Obj. 3. In previous assays with the Black cutworm (BCW), ***Agrotis ipsilon***, the relative growth rate of 2nd instar black cutworm was lower when fed on maize leaves from endophytic plants compared to control plants where plants were grown under conditions of adequate water (Ahmad et al. 2020). In designing experiments for this objective, we had planned to determine the relative and interacting effects of water stress and endophytic ***M. robertsii*** on BCW relative growth rate. However, even though corn plants in this greenhouse experiment were caged individually, we were not able to recover all of the BCW that we had applied, even from plants where feeding damage was evident. Therefore, our discussion is based on plant damage from BCW feeding, rather than effects on BCW growth.

Unexpectedly, frequency and severity of damage from BCW feeding in the Adequate, but not Deficit or Excess, water treatments, was greater in corn inoculated with ***M. robertsii*** than in the uninoculated control. These unexpected results may be due to changes in expression of stress-related genes and related plants chemistry. We collected foliar and root material from the plants used in this experiment to determine the effects of the treatments on plant molecular and phytochemical plant defenses (Barbercheck et al. 2019). We are currently examining ways in which plants Water stress may have impaired plant defenses so that damage frequency was similar in plants stressed by water deficit or excess. In relatively unstressed plants in the Adequate water treatment, ***M. robertsii*** may have "turned off" plant defenses to allow endophytic colonization, which may help explain the greater frequency and severity of damage in the ***Metarhizium*** treated vs uninoculated plants (Thaler et al. 1999). We are analyzing expression of corn defense genes related to water stress, insect feeding, and infection by plant pathogens to determine the mechanism underlying this response.

Key points and conclusion

- Approach to tillage (inversion, shallow tillage, and no-till) did not affect the season-long prevalence of ***Metarhizium robertsii*** in soil indicating that judicious use of tillage will not eradicate populations of this beneficial fungus.

- Corn seed inoculated with ***M. robertsii*** and planted in the field did not result in colonization of plants by ***M. robertsii*** suggesting that ***M. robertsii***, at least the strain that we used, is a relatively poor competitor with other rhizosphere or endophytic microbes. In the short-term, ***Metarhizium*** could be used as a soil inoculant where growth media with low microbial abundances are used and water is managed, for example, in greenhouse production of seedlings or transplants.
- In the field, soil moisture and clay content are key factors positively influencing, and soil S negatively influencing, the prevalence of ***M. robertsii***. While clay content is not easily manageable, soil moisture and S are manageable.
- Excessive soil moisture results in lower endophytic colonization of corn by ***M. robertsii*** compared with adequate or deficit soil moisture, most likely due to microbial degradation of spores and high S concentrations. Soil and plant S, even within normal agronomic ranges, appear to be detrimental to ***M. robertsii***.
- In plants under water stress, ***M. robertsii*** may have detrimental, rather than beneficial effects on plant performance. The conditions under which beneficial endophytes may themselves become stressors will be an important topic for future research, especially with increased environmental plant stress predicted with climate change.

7. Outreach:

Because of restrictions to in-person events and activities related to COVID over the last two years, the majority of outreach efforts associated with this project were virtual. The materials and activities listed below incorporated information that we learned from project activities. We will continue to incorporate project-generated information into extension and scientific articles and presentations. Extension and research outreach publications and activities that were accomplished during the funding period targeted scientists, farmers and agricultural professionals.

Outreach to Farmers and Agricultural Professionals

Barbercheck, M. 11 March 2022. Soil health research update. Central Susquehanna Organic Growers Network meeting. New Columbia, PA. 25 attendees.

Barbercheck, M. 2022. Better pest management through soil health. Webinar. Virtual Crops Conference. 3 March 2022. 34 attendees

Barbercheck, M. 2022. Soil health and IPM. Perry Co. Corn Day. 28 Feb. 2022. 64 attendees

Barbercheck, M. 2022. Organic pest management in grains with a focus on noctuids. OGRAIN Virtual Conference, University of Wisconsin. 4 February 2022.

Barbercheck, M.E. 2021. Penn State Organic Research Update. Rodale Reduced Tillage Webinar Series. 235 attendees. 3 February 2021.

Barbercheck, M., L. Blazure (Organizers). 2021. Advanced Soil Ecology for Meeting Crop Health and Environmental Goals. 478 registered. Five-part webinar series:

1. Session 1 (Blazure; March 4, 1:00-2:00 pm EST): Soil Bacteria: Small but mighty (280 viewers).
2. Session 2 (Blazure; March 11, 1:00-2:00 pm EST): Mycorrhizal Fungi: Networking for plant health (186 viewers).
3. Session 3 (Barbercheck; March 18, 1:00-2:00 pm EST): Endophytes: Hidden helpers in plants (178 viewers).
4. Session 4 (Barbercheck; March 25, 1:00-2:00 pm EST): Soil Food Web: Where the hunters become the hunted (127 viewers).
5. Session 5 (T. Bell; April 1, 1:00-2:00 pm EST): Soil microbiome: The present and future of boosting soil health through microbial management.

Barbercheck, M. (panelist with J. Wallace, K. Borrelli, J. Cook). 2020. Organic Crop Production Q & A. Virtual Ag Progress Days Session. 34 attendees. 11 August 2020.

Barbercheck, M. (organizer and presenter) 2020. Organic Research Flash Talks & Discussion Session. PASA Farming for the Future Conference, 8 Feb. 2020. 80-min workshop. Lancaster, PA. 20 attendees

Barbercheck, M. 2020. Pest Management Strategies for the Transitioning Producer (Invited). American Society of Agronomy "Managing through the Organic Transition in Grain Crop Production webinar series. https://www.agronomy.org/education/classroom/classes/658

Barbercheck, M. 2020. Fantastic endophytic fungi: What they are, what they do, and how to conserve them. (Invited). Conservation Tillage Conference. March 4, 2020. Ohio Northern University. Ada, Ohio. 200 attendees

Extension publications and articles

Barbercheck, M., Borrelli, K. (eds.). 2021. Penn State Organic Crop Production Guide. # AGRS-124G. https://extension.psu.edu/penn-state-organic-crop-production-guide

Barbercheck, M. 2020. Many Factors Influence Interpretation of Soil Health Tests. Field Crop News, 15 May 2020. https://extension.psu.edu/many-factors-influence-interpretation-of-soil-healthtests?j=536510&sfmc_sub=35519620&l=159_HTML&u=10724487&mid=7234940&jb=9&utm_medium=email&utm_source=MarketingCloud&utm_campaign=FAFC-2020-MAY-13-GN-EM-Field+Crop+News&utm_content=FAFC-2020-MAY-13-GN-EM-Field+Crop+News&subscriberkey=0030W00003P0ySiQAJ

Scientific presentations

Peterson, H., Barbercheck, M. 2022. Impact of Water Stress on the Establishment and Persistence of Endophytic and Entomopathogenic ***Metarhizium robertsii***. Eastern Branch ESA Meeting, April 2022 Philadelphia, PA

Barbercheck, M, Ahmad, I. 2022. Going underground: Ecology of a multifunctional fungus in organic cropping systems. Invited seminar, 10 Jan. 2022. NCSU Dept. of Entomology and Plant Pathology.

Ahmad I., Jiménez-Gasco, M.D.M., Luthe, D., Barbercheck, M. 2020. Mighty Microbes: The tri-trophic interactions of endophytic ***Metarhizium*** in maize. XXVIII Plant and Animal Genome, 2020, San Diego, CA, USA, Jan. 11-15, 2020.

Barbercheck, M.E., Regan, K., Rivers, A., Voortman, C. 2020. Ground rules: Conserving epigeal predators and other beneficial organisms in organic cropping systems. Invited talk for Symposium "Insect pests and beneficial arthropods in climate-change-resilient diversified cropping systems". Entomological Society of America Annual meeting, Orlando, FL. Nov. 2020

Scientific publications

Flonc, B., Barbercheck, M., Ahmad. I. 2021. Observations on the relationships between endophyti***c Metarhizium robertsii*** and ***Spodoptera frugiperda*** (Lepidoptera: Noctuidae) on maize. Pathogens 10(6), 713. https://doi.org/10.3390/pathogens10060713. Special Issue Plant-Microbe-Invertebrate Pest Interactions. https://www.mdpi.com/2076-0817/10/6/713

Ahmad, I., M.d.M. Jimenez-Gasco, M. Barbercheck. 2020. The Role of Endophytic Insect-Pathogenic Fungi in Biotic Stress Management, Ch. 13 (pp. 379-400) in: B. Giri, M. P. Sharma (eds.), Plant Stress Biology, Springer Nature Singapore. https://link.springer.com/chapter/10.1007%2F978-981-15-9380-2_13

Ahmad, I., M. del M. Jiménez-Gasco, D. S. Luthe, M. E. Barbercheck. 2020. Systemic colonization by ***Metarhizium robertsii*** enhances cover crop growth. Journal of Fungi 62(2):64 https://doi.org/10.3390/jof6020064

Ahmad, I., M. Jiménez-Gasco, D. S. Luthe, S.N. Shakee, M.E. Barbercheck. 2020. Endophytic ***Metarhizium robertsii*** promotes maize growth and suppresses insect growth by eliciting plant defense. Biological Control 144: https://doi.org/10.1016/j.biocontrol.2019.104167

Cloutier, M., E. Murrell; M. Barbercheck; J. Kaye; D. Finney; I. Garcia-Gonzalez; M. A Bruns. 2020. Fungal community shifts in soils with varied cover crop treatments and edaphic properties. Scientific Reports 10: 6198. https://doi.org/10.1038/s41598-020-63173-7

8. Financial accounting

The Excel spread sheet is attached as a separate document. All of the funds were expended.

Variances in expenditures occurred. We expended less than budgeted for:
Personnel costs and Fringe (wage payroll, expended less than budgeted), travel and mileage, materials and supplies and greenhouse use. A portion of these lines were charged to Barbercheck et al. (2019) for activities and materials fulfilling objectives common to both projects. I used my personal vehicle for 6 out of 9 farm visits to collect soil samples and did not charge the grant.

We expended more than budgeted for:
Soil analysis. We conducted much more extensive soil analysis than anticipated when creating the original budget, including 9 Cornell CASH tests ($130 per sample) to support interpretation of results and to help accommodate collaborating farmer interests. Data from soil analyses were essential in understanding and interpreting the results of each objective.

This project received additional funding to expand the scope of the research:
Barbercheck, M., Jimenez-Gasco, M.d.M., Felton, G., Ahmad, I. 2019. Conservation of a Multifunctional Fungus for Plant Protection in Organic Cropping Systems USDA ORG Award # 2019-51106-30198

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Ahmad, I., M. Jiménez-Gasco, D.S. Luthe, S.N. Shakeel, M.E. Barbercheck. 2020. Endophytic ***Metarhizium robertsii*** promotes maize growth and suppresses insect growth by eliciting plant defense. Biological Control 144: Doi: 10.1016/j.biocontrol.2019.104167

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Barbercheck, M., Jimenez-Gasco, M.d.M., Felton, G., Ahmad, I. 2019. Conservation of a Multifunctional Fungus for Plant Protection in Organic Cropping Systems USDA ORG Award # 2019-51106-30198

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