A SURVEY OF *FUSARIUM OXYSPORUM* IN GEORGIA HEMP FARMS AND VEGETABLE GARDENS

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Recent changes in U.S. federal law have recognized the genetic and chemical differences between hemp and marijuana **(***Cannabis sativa* L.), resulting in the legalization of hemp in the United States. The barriers to this plant's growth in the state of Georgia are not well understood due to its prohibition for the past 80 years. Among the many possible obstacles is fungal disease. *Fusarium oxysporum* (Schlecht. emend. Snyder & Hansen) is a fungus that damages various crops and was previously researched as a biocontrol agent for *C. sativa*. Thus, this study surveyed hemp farms in Georgia to determine the prevalence of *F. oxysporum* in native soils via qPCR and correlate their abundance with the physicochemical properties of the soil (soil moisture, pH, ammonium, nitrate, and total inorganic nitrogen). Home vegetable gardens were also surveyed to be compared to the *F. oxysporum* abundance of hemp farms. We found that 5 out of 20 of the hemp soil samples and 1 out of 8 of the vegetable garden samples tested positive for *F. oxysporum*. Correlation analysis revealed a strong negative correlation between *F. oxysporum* abundance and soil moisture (r = -0.850, P=0.031). Although there were several plants reported to have symptoms matching that of fusarium wilt, no hemp farmer reported a substantial loss of crops from fungal disease. Overall, the findings of this study suggest that *F. oxysporum* may not pose a significant barrier to hemp farming in Georgia.

INTRODUCTION

Cannabis sativa L. (hemp, marijuana) is a crop that provides a wide variety of medicinal, industrial, and environmental usages (Sarfaraz et al., 2005; Rodriguez-Leyva & Pierce, 2010; Esposito et al., 2013; Salentijn et al., 2015; Campos et al., 2016; Harper et al., 2018; Turner et al., 2019; Wu et al., 2021). The history of its legality in the United States is complicated, first being regulated in the Marijuana Tax Act of 1937 and outlawed for all usage in the Controlled Substance Abuse Act of 1970 (Belenko, 2000). Recent changes in U.S. federal law have recognized the genetic and chemical differences between hemp and marijuana, resulting in the development of hemp-growing pilot programs by the 2014 Farm Bill and, eventually, the legalization of hemp in the 2018 Farm Bill (Tyler et al., 2020). Although members of the same species, *C. sativa* plants with a dry weight of (–)-trans-Δ^9-tetrahydrocannabinol less than or equal to 0.3% is recognized as hemp; any plant with a concentration over 0.3% dry weight is considered marijuana. This legal distinction led to the rapid expansion of hemp production, with a reported 146,065 planted acres in 2019 in the U.S. (Tyler et al., 2020). A report by the United States Department of Agriculture found that the value of hemp production for the U.S. in 2021 totaled $824 million (USDA, 2022), making it an important economic product.

Among the challenges facing this industry are fungal pathogens, which can infect the plant during multiple phases of production, including after harvesting. For example, species of *Botrytis* are known to cause bud rot, which generates significant losses during the storage of hemp products, and species of *Pythium* cause decay in hemp roots, stunting the growth of the plant (Punja et al., 2019). *Fusarium oxysporum* (Schlecht. emend.

Snyder & Hansen) can cause rot of the bud, stem, crown, and root of *C. sativa* plants (Punja, 2021a, 2021b; Punja et al., 2018; Punja & Ni, 2021) and is the most economically important species complex of *Fusarium* (Lombard et al., 2019), causing vascular wilt of many plant species. Certain *formae speciales* of *F. oxysporum* are so damaging to *Cannabis* growth that in the past, strains like *F. oxysporum* f. sp. *Cannabis (FoxC)* have been researched as biocontrol agents for the plant. Mortality rates of *FoxC* are as high as 50%, and the most infectious strains have been observed to infect 50.0-66.7% of the plants in a field over the course of six months (Hildebrand & Mccain, 1978; McCain & Noviello, 1984; Tiourebaev et al., 2001). Additionally, some isolates of *F. oxysporum* have been found to produce mycotoxins (beauvericin, bikaverin, enniatin B, and moniliformin) (O'Donnell et al., 2018), which render some products harmful to human consumption (Prosperini et al., 2013; Kalayou et al., 2015; Knutsen et al., 2018; Mallebrera et al., 2018). Furthermore, phytopathogenic *Fusarium* species may also remain in soils after infection due to the formation of thick-walled and lysis-resistant chlamydospores (Griffiths 1973); this can make them a chronic threat to farmers.

Recently, a study in North Carolina that surveyed the potential diseases of hemp found that 17% of the hemp samples collected in 2018 were infected with *F. oxysporum* (Thiessen et al., 2020). Georgia has little hemp production compared to North Carolina, with a reported 130 acres of hemp planted in Georgia versus 2,150 acres in North Carolina (USDA, 2022). As a result, the concentrations of *F. oxysporum* in the soil of Georgia hemp farms are unknown; whether or not it poses a significant barrier to the expansion of Georgia hemp production is also unknown. Previous research has established a real-time quantitative polymerase chain reaction (qPCR) assay capable of quantifying pathogenic and nonpathogenic *F. oxysporum* populations in soil by amplifying the nrDNA ITS region of DNA (Mishra et al., 2003; Jiménez-Fernández et al., 2010). The present study thus sought to survey hemp farms in Georgia and quantify *F. oxysporum* gene copy numbers in their native soils, using home vegetable gardens as a comparison. In addition, physiochemical parameters such as soil moisture, pH, ammonium (NH_4^+), nitrate (NO_3^-), and total inorganic nitrogen (TN = NH_4^+ + NO_3^-) were measured to better understand the relationship between *F. oxysporum* presence and soil characteristics. The measurement of soil nitrogen was specificly selected for the current study because numerous studies have shown that soil nitrogen levels can significantly effect *F. oxysporum* presence and subsequent plant wilt. For example, previous research conducted in China indicates that organic nitrogen application (90 kg ha^{-1} of N) can decrease Fusarium wilt in faba bean plants (Lv et al., 2021). In addition, there is evidence that NO_3^- fertilizers reduce Fusarium wilt while NH_4^+ increases the severity of wilt (Wang et al., 2016; Zhou et al., 2017).

MATERIALS & METHODS

Soil Sample Collection

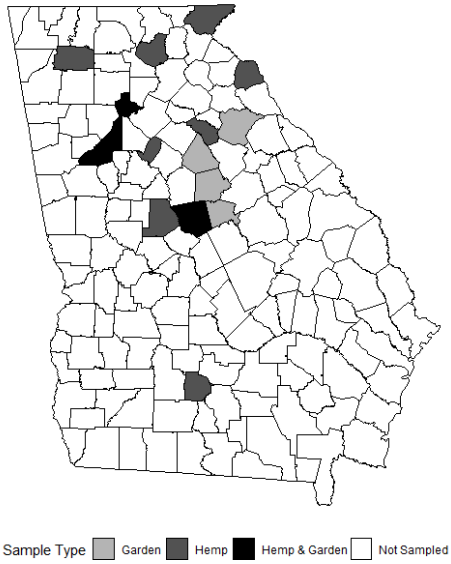

Figure 1. The locations of the collected soil samples and the sample type.

A list of licensed hemp growers was acquired from the University of Georgia, and growers were contacted via email requesting their participation in the study. Of the 120 contacted growers, 10 hemp farms were selected for soil collection based on the grower's willingness to participate, their location to other hemp farms, and their use of native soils. All hemp farms had less than five total acreas of cultivated soil. Hemp varities at all farms were grown for the extraction of legal cannabinoids, such as cannabidiol (CBD) and cannabigerol (CBG), and/or the production of flower parts for smoking. The majority of hemp farms that were visited had plants in the flowering stage of growth. To the best of our knowledge, industrial hemp was not grown at any site. Soil was also surveyed from traditional gardens located at eight different residences. Gardens were selected as a comparison group because of similar cultivation practices and scale. In addition, gardens in Georgia typically have plants (tomatoes, melons, and squash) that are susceptible to *F. oxysporum*. All soil samples were collected during the month of August 2021.

Approximately 500 g of soil was collected from the top 10 – 15 cm of each hemp or garden plot.. The 500 grams of soil collected from each plot was a composite sample that represented atleast 15 different locations within the growing area. All soils were collected from the base of a plant or between adjacent plants. If growers reported using different "growing zones" or soil types, multiple composite samples were taken from select farms. Specifically, three separate composite soil samples were collected from Walton County and Oconee County hemp farms. In addition, two different composite soil samples were collected from a hemp farm located in Hart County. The soil was retrieved from each plot by hand using sterile nitrile gloves and placed into a sterile bag. Soils were thoroughly mixed by hand in the bag. All samples were stored on ice during transportation to the lab. Approximately half of each soil sample was air-dried for 48 hours and passed through a 2-mm sieve. Air-dried soil was used to measure pH,water-extractable $NH_4^{+,}$ and water-

extractable NO_3^-. Ten grams of moist soil was used for water content determination before the remaining soil was stored at 3°C.

Determination of Soil Characteristics and Nutrients

Water-extractable NH_4^+ and NO_3^- of each soil were determined by shaking 1 g of soil with 100 ml of dH_2O for 5 minutes; after shaking, the solution was filtered through a 0.45-µm filter, and the filtrate was measured using a GoDirect NH_4^+ Ion-Selective Electrode and a GoDirect NO_3^- Ion-Selective Electrode (Vernier, Beaverton, OR). The soil pH of each sample was measured in a 1:1 ratio in 0.01 M $CaCl_2$ (Miller & Kissel, 2010). Soil water content was determined gravimetrically by measuring the samples before and after air drying at 105°C for 48 hours (Campbell & Campbell, 2013).

DNA Extraction

The soil samples were air-dried and sieved using a 2-mm sieve. Soil community DNA was extracted from 250mg of each soil using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) in duplicates for each soil sample. The duplicate soil extractions were combined in a microcentrifuge tube, and the DNA was concentrated in an Eppendorf Vacufuge Plus at 30°C for 6 mins. DNA concentration (ng μl^{-1}) of each sample was determined using a Nanodrop ND-1000 Spectrophotometer (ThermoFisher, Waltham, MA) before storing at -20°C.

Potato dextrose agar plates of nine different isolates of *F. oxysporum* from various geographical locations (Florida and Los Angeles, USA; Malawi; Netherlands; Israel) were provided by the Brewer mycology lab at the University of Georgia. These plates were stored at 4°C until their DNA was extracted. A pure culture of *Escherichia coli* (ACCT: 10798) was also prepared in Tryptic Soy Broth to be used as a negative control in qPCR assays (Sigma-Aldrich, St. Louis, MO). DNA was extracted from small pieces of agar growing *F. oxysporum* and a 100µl aliquot of *E. coli* using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany). DNA concentration (ng μl^{-1}) of each sample was determined using a Nanodrop ND-1000 Spectrophotometer (ThermoFisher, Waltham, MA) before storing at -20°C.

qPCR Assay of Fusarium oxysporum

The FOF-1 (5`-ACATACCACTTGTTGCCTCG-3`) and FOR-1 (5`-CGCCAATCAATTTGAGGAACG-3`) primers for the *F. oxysporum* ITS region were obtained from Mishra et al. (2003). Reactions with a final volume of 20 µl were prepared with 50-100 ng of extracted DNA, 500 nM of each primer, 10 µl of SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA), and 1.5 mM of $MgCl_2$. A Bio-Rad CFX96 (Bio-Rad, Hercules, CA) was used to run the qPCR reactions. All qPCR standards, controls, and soil samples were run in duplicate for each reaction which is similar to other environmental studies (Rothrock et al., 2008; Burt et al., 2021; Truitt et al., 2022; Chapman et al., 2023). Extracted *E. coli* DNA and a reaction mixture with no DNA were used as negative templates in every assay run; during all PCR assays, the negative control did not amplify.

A temperature gradient cycling program was run to determine the optimal annealing temperature of the assay, but other than the annealing temperature, the

parameters were as described by Jiménez-Fernández et al. (2002). The temperature gradient ranged from 60°C to 70°C, and the assay used extracted *F. oxysporum* DNA as a positive template. The optimal annealing temperature on our instrument was determined to be 61°C.

To confirm the qPCR assay worked on various *F. oxysporum* isolates, DNA extracted from the nine *F. oxysporum* agar plates was tested using the abovementioned parameters. These isolates included two from Israel (NRRL 36570 and 36464), one from Malawi (NRRL 25609), two from the Netherlands (NRRL 26960 and 26961), two from Los Angeles, US (NRRL 34076 and 34049), and one from Florida, US (NRRL 26379). After the reactions, the well with the lowest C_q value was purified using an ssDNA/RNA Clean & Concentrator (Zymo Research, Irvine, CA). DNA concentration (ng μl^{-1}) of the purified product was determined using a Nanodrop ND-1000 Spectrophotometer (ThermoFisher, Waltham, MA), and gene copy number was calculated based on the 340 bp product size; standards at 10^8 to 10^3 gene copy numbers were generated by serial dilution.

DNA extracted from each soil sample was run in duplicate using the described assay parameters. A standard curve with an r^2 value of 0.99 was generated using the serially diluted qPCR productto quantify the gene copy numbers of each soil sample.

Statistical Analysis

Statistical analysis was performed using the R programming language (R Core Team, 2017). A two-tailed Student's t-test was used to determine whether data sets differed significantly. The Shapiro-Wilks test and Levene's test were used to test the assumptions of Student's t-test. A chi-squared test was performed to analyze differences in *F. oxysporum* detection frequencies in soil samples from hemp farms and soil samples from vegetable gardens. Point biserial correlations were used to determine the correlation between physicochemical soil parameters and the detection of *F. oxysporum*. Pearson's correlation analysis was used on the positive subset to determine linear relationships between the physicochemical soil parameters and the *F. oxysporum* log gene copy number (FoLGC). For all correlations, soil pH was excluded from the analysis due to the relationship between *F. oxysporum* growth and pH not being strictly linear and dependent on several other factors, mainly temperature (Cruz et al., 2019). Statistical differences were determined using Fisher's protected LSD at $p = 0.05$.

RESULTS

Twenty-eight soil samples were collected from around Georgia, 20 from hemp farms and 8 from vegetable gardens. Out of these samples, 6 of them had detectable ITS gene counts for *F. oxysporum* (greater than the detection limit of 10^4) (Table I). Five positives came from hemp samples (25% Hemp Samples), and 1 was from a vegetable garden (12.5% Vegetable Gardens). Three positive hemp samples originated from the same site in Walton County. A chi-squared test determined that detection frequencies between hemp and garden soil samples were not significantly different (X^2 (1, $N = 28$) = 0.5303, $p = 0.4665$).

Table I. The location, *Fusarium oxysporum* log gene-copy number, and soil parameters for each sample that tested positive for *Fusarium oxypsorum*.

Georgia County	Site Type	Log Gene Copy #	Soil Moisture	Soil pH	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	Total Inorganic N (mg kg^{-1})[†]
Hart	Hemp	4.62	31.40%	6.49	13.60	64.24	77.84
Walton	Hemp	5.79	3.90%	5.37	9.60	128.38	137.98
Walton	Hemp	4.38	26.15%	6.82	8.88	103.12	112.00
Walton	Hemp	5.92	3.83%	5.91	4.00	39.52	43.52
Tifton	Hemp	5.57	6.92%	5.29	1.84	5.28	7.12
Baldwin	Garden	4.62	11.83%	7.52	5.76	22.96	28.72

[†]Total Inorganic N was calculated by adding NH_4^+ and NO_3^- concentrations.

Table II. Mean values and Student's t-test for soil parameters of the samples that tested positive for *Fusarium oxysporum* compared to the samples that tested negative.

Soil Parameter	*F. oxysporum* (+) Mean	*F. oxysporum* (-) Mean	*t*-Statistic	*df*	*p*-Value
Soil Moisture (%)	14.0 ± 11.9	21.8 ± 21.6	0.66113	8.0671	0.53
Soil pH	6.23 ± 0.872	6.00 ± 0.723	-0.61827	6.8183	0.56
NH_4^+ (mg kg^{-1})	7.28 ± 4.26	8.05 ± 5.81	0.37044	10.062	0.72
NO_3^- (mg kg^{-1})	60.6 ± 47.1	41.6 ± 52.7	-0.85888	8.2246	0.41
Total Inorganic N (mg kg^{-1})	67.9 ± 50.6	49.6 ± 57.7	-0.77055	8.4403	0.46

There were no significant differences in any soil parameters between the samples with detected *F. oxysporum* and the samples with no detected *F. oxysporum* (Table II).

Table III. Point biserial correlation between measured soil parameters and the detection of *Fusarium oxysporum* or lack thereof.

Soil Parameter	Point Biserial Correlation
Soil Moisture	0.142
NO_3^-	-0.201
NH_4^+	-0.021
Total Inorganic N	-0.187

Point biserial correlation for physicochemical soil parameters and the detection of *F. oxysporum* was calculated (Table III). This analysis revealed no significant correlations between the detection of *F. oxysporum* and soil parameters.

Table IV. Pearson's correlations between *Fusarium oxysporum* log gene-copy number and measured soil parameters among the positive samples.

Soil Parameter	Pearson's *r*	*P*-Value
Soil Moisture	-0.850	0.031
NO_3^-	0.061	0.907
NH_4^+	-0.483	0.332
Total Inorganic N	-0.099	0.853

A Pearson correlation analysis between FoLGC and physicochemical soil parameters of the positive subset revealed that soil moisture (r = -0.850) correlates negatively with FoLGC.

Table . Mean values and Student's *t*-test between hemp soil samples and garden soil samples.

Soil Parameter	Hemp Mean	Garden Mean	*t*-Statistic	*df*	*p*-Value
Soil Moisture (%)	15.46 ± 11.5	20.9 ± 15.35	0.845	8.912	0.42
Soil pH	5.77 ± 0.68	6.74 ± 0.61	3.444	12.436	0.0046
NH_4^+ (mg kg^{-1})	7.39 ± 5.26	6.45 ± 1.69	-0.664	21.443	0.514
NO_3^- (mg kg^{-1})	54.31 ± 48.74	24.70 ± 17.23	-2.194	21.826	0.039
Total Inorganic N (mg kg^{-1})	61.7 ± 53.2	31.14 ± 17.59	-2.105	21.568	0.047

Student's *t*-test was conducted to determine differences between soils used in hemp farms and vegetable gardens. This analysis revealed that the soils in hemp farms and vegetable gardens were significantly different in soil pH, water-extractable NO_3^-, and total inorganic nitrogen ($p < 0.05$) (Table V). The average pH of the hemp soil samples (5.77 ± 0.68) was significantly less than the garden samples (6.74 ± 0.61) (p = 0.0046), and the water-extractable NO_3^- (54.31 ± 48.74 mg kg^{-1}) and total inorganic nitrogen of the hemp samples (61.7 ± 53.2 mg kg^{-1}) were significantly greater than the NO_3^- (24.70 ± 17.23 mg kg^{-1}) (p = 0.039) and total inorganic nitrogen (31.14 ± 17.59 mg kg^{-1}) (p = 0.047) of the garden samples. The NO_3^- and total inorganic nitrogen of the hemp samples were also much more variable than that of the vegetable garden samples, having standard deviations more than twice those of the gardens (Table V).

Discussion

Fusarium oxysporum is a fungal species complex known to contain both pathogenic and non-pathogenic strains. The pathogenic members of this species complex are known to have both animal and plant hosts, but research is mainly focused on the vascular wilt it causes in plants (Dean et al., 2012). *Fusarium oxysporum* strains that infect *C. sativa* have been investigated as a mycoherbicide for the plant and are an effective form of biological control against the plant (Hildebrand & Mccain, 1978; McCain & Noviello, 1984; Tiourebaev et al., 2001). Given that *C. sativa* was only recently legalized for growth in the United States, we sought to determine how abundant *F. oxysporum* is in the soil of hemp farms, using the soil of vegetable gardens as a comparison. *Fusarium oxysporum* was detected in 25% of soil samples collected from hemp farms and in 12.5%

of the soil samples from vegetable gardens. A chi-squared test determined that there was not a significant difference in detection frequencies of *F. oxysporum* in the soil of hemp farms and vegetable gardens (X^2 (1, N = 28) = 0.5303, p = 0.4665). Our results are somewhat suprising because numerous studies state that *F. oxysporum* is ubiquitous in the environment (Shabani et al., 2014; Arie, 2019; Srinivas et al., 2019)

Not all sites with soil positive for *F. oxysporum* had crops that exhibited signs of Fusarium wilt. Personal correspondence with the grower at the Walton County farm after the season ended indicated that although a few plants exhibited symptoms consistent with Fusarium wilt, there was no significant loss due to disease. In addition, one of the two hemp plots at the Hart County farm tested positive for *F. oxysporum* (Table 1), and the grower stated that a "few plants" were lost in this area due to a fungal disease resembling wilt. Thus, only four of the five positive hemp soil samples (20% of hemp soil samples) were taken from sites with plants that purportedly exhibited symptoms consistent with Fusarium wilt; however, the presence of *F. oxysporum* in plant material can not be confirmed at this time It should be noted that plant material from these farms was not tested for the presence of *F. oxysporum* because hemp is a controlled substance, and the researchers involved in this project did not have permission to remove plant material from farms and store in university research space. Our results are similar to Thiessen et al. (2020), which surveyed diseases of *C. sativa* plants in North Carolina to determine agricultural threats to industrial hemp production. During the survey, 17% of the samples collected in 2018 exhibited fusarium wilt. However, the present study differs from Thiessen et al. (2020) in that the soil surrounding hemp plants was sampled rather than the plant itself, and in the present study, healthy and diseased sites were sampled rather than only diseased plants. Based on our results and the lack of significant crop loss among the 10 farms, it is reasonable to believe that *F. oxysporum* does not pose a significant threat. In addition, our qPCR primers used to detect the presence of *F. oxysporum* do not differentitate between saprophytes and plant pathogens. Therefore, it is possible that the *F. oxysporum* that was detected at select sites was not pathogenic to hemp, and this could explain the lack of fungal disease that was observed. More extensive studies can be undertaken that sample both soil and plant material from hemp farms, but at this time it does not appear that *F. oxysporum* represents a significant threat to hemp production in Georgia.

Numerous studies have found that high soil moisture can inhibit *F. oxysporum* growth over time (Stover, 1953; Park, 1958; Newcombe, 1960; Oritsejafor, 1986). These studies suggest that saturated soils' carbon dioxide accumulation and low oxygen tension stress the fungus when nutrients are limited, decreasing biomass over time (Oritsejafor, 1986). Nutrient limitation is critical for inhibiting *F. oxysporum* growth in environments with high soil moisture, with at least two studies reporting that *F. oxysporum* growth is not significantly inhibited by the lack of oxygen alone (de la Broise & Durand, 1989; Aguilar et al., 1998). In the present study, soil moisture for *F. oxysporum* positive samples was found to have a strong negative correlation with FoLGC (r = -0.850) (Table 4). This study differs from previous literature in that it uses measurements of soil moisture and FoLGC from singular time points and has not eliminated confounding variables. Additionally, it is unlikely that the sampled soils are limited in nutrients because they are in close proximity to actively growing agricultural products. So, although low oxygen can contribute to decreased *F. oxysporum* growth when nutrients are also limited, our study

did not meet these conditions. Thus, this study reports novel findings concerning the correlation of *F. oxysporum* detection with soil moisture.

In this study, the physicochemical soil properties of vegetable gardens and hemp farms differed significantly in pH (p = 0.0046), NO_3^- (p = 0.039), and total inorganic nitrogen (p = 0.047) (Table 5). Specifically, the average soil pH of the hemp samples (5.77 ± 0.68) was more acidic than that of the vegetable garden samples (6.74 ± 0.61). The average soil pH for the hemp samples is consistent with the findings of Caplan et al. (2017), which observed the pH range of 5.1-7.4 to be acceptable for cannabis growth; similarly, the average soil pH of the vegetable gardens is consistent with the pH of ~6.5 that is suggested by popular consumer resources (Almanac, 2022). Additionally, studies have found that increasing soil NO_3^- increases hemp biomass (Vera et al., 2004; Yang et al., 2021) so long as the NO_3^- concentration does not exceed about 155 mg kg^{-1} (Yang et al., 2021). The NO_3^- measurements for the hemp samples in this study are within that range (54.31 ± 48.74 mg kg^{-1}) but vary significantly. The average soil nitrate for the garden samples (24.70 ± 17.23 mg kg^{-1}) is significantly less than that of the hemp samples and varies less drastically. The wide range of soil NO_3^- concentrations in the hemp samples may be due to hemp being a relatively recent agricultural product in Georgia, with common practices for cultivation not being widespread yet. Finally, the fact that there was significantly greater total inorganic nitrogen in the hemp samples (61.7 ± 53.2 mg kg^{-1}) than in the garden samples (31.14 ± 17.59 mg kg^{-1}) is driven by the significantly greater nitrate concentration.

To conclude, this study surveyed soil from hemp farms and vegetable gardens across the state of Georgia. Of these samples, *F. oxysporum* was detected in 25% of hemp farm samples and 12.5% of vegetable garden samples. However, only 4 of the hemp samples were taken from sites that had plants displaying symptoms consistent with fusarium wilt; however,there are many fungal diseases that cause bud rot in cannabis plants. Even though *F. oxysporum* has been described as being widely distributed in the environment, it seems reasonable to conclude that *F. oxysporum* does not currently pose a significant threat to Georgia hemp production. Additionally, among the subset of samples that tested positive for *F. oxysporum*, we found FoLGC to be negatively correlated with soil moisture (r = -0.850); this trend is new to the literature and should be confirmed with research focused on optimal parameters for *F. oxysporum* growth in soil. Finally, we found that in Georgia, the physicochemical soil parameters of vegetable gardens and hemp farms are significantly different, specifically in soil pH, NO_3^- concentration, and total inorganic nitrogen.

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