Roles of native *Trichoderma* isolates in reducing tomato *Fusarium* wilt and increasing phosphorus uptake

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ABSTRACT

Beneficial soil fungi, like *Trichoderma spp.* isolates, can parasitize structures of plant pathogenic fungi, solubilize poorly-soluble soil nutrients such as phosphorus (P), and induce crop resistance to plant pathogens, however, little information exists on the magnitude of Trichoderma benefits for plant health and production. This study evaluated eight native *Trichoderma* isolates—*T. afroharzianum*, *T. arundinaceum*, *T. asperelloides*, *T. asperellum*, *T. hamatum*, and several *T. harzianum* species complex isolates—for ability to reduce *Fusarium oxysporum* f. sp. *lycopersici (Fol*) and to solubilize forms of soil P (aluminum or calcium phosphates). This work was conducted as four separate objectives and we hypothesized that effects of *Trichoderma* on Puptake and reduced wilt incidence caused by *Fol* would vary among indigenous *Trichoderma* isolates and tomato germplasm across a tomato domestication gradient and significant correlations would be a result of *Trichoderma*-induced effects on P-uptake and disease reduction in tomato. Chapter 2, objective 1, assessed the ability of eight *Trichoderma* isolates (T1 to T8) to reduce *Fol* in tomato (*Solanum lycopersicum* cv. Valencia). *Trichoderma* treatments increased biomass and reduced disease symptoms compared to controls; T3 and T5 were most effective. Objective 2 assessed *Trichoderma* potential to solubilize secondary P compounds in Pikovskaya's (PVK) broth and competitive interactions with *Fol* in lab assays on PVK agar. Isolate T8 showed the highest biomass in P-deficient conditions, and isolates T5, T6, T7, and T8 exhibited competitive interactions with *Fol* on aluminum phosphate. In Chapter 3, Objective 1, Trichoderma isolates T3 (*T. arundinaceum*) and T5 (*T. harzianum*) were screened for the potential to solubilize soil P and increase crop uptake in genetically diverse and varying domesticated tomatoes. The effect of *Trichoderma* on P uptake was limited and varied by tomato genotype and P source. In Objective 2, the impact of *Trichoderma* (T5) on P solubilization and *Fol* resistance was studied in genetically diverse tomatoes. *Trichoderma* improved aluminum phosphate solubilization, and tomatoes with aluminum phosphate fertilization showed greater resilience and lower disease ratings. This research concluded that the effectiveness of *Trichoderma* in improving crop growth and disease resistance depends on the soil chemical environment, *Trichoderma* isolate traits, and tomato genotype.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Tomato Production

Solanum is a large and diverse genus of flowering plants in the *Solanaceae* family, with an estimated 1,250 – 1,700 species (Bergougnoux et al., 2013). Many of these plants are recognized as "nightshades" as they contain medicinal and/or poisonous compounds with native plants in all temperate and tropical regions. The most important crop species of the genus *Solanum* include *S. tuberosum* (potato), *S. melongena* (eggplant), *and S. lycopersicum* (tomato). *Solanum* is recognized for its morphological and ecological diversity and is considered the most economically important genus (Bergougnoux, 2013). The name tomato is derived from the Mexican word "tomatl" with the Andean zone being the center of origin of the wild tomato (Kalloo et al., 1991). The first domestication practices of tomato began in South America (Fuentes et al., 2021), and plants were taken to Europe for continued cultivation in the 15th century (Bai et al., 2007). Over time, these practices have allowed for a huge variety of morphologically different cultivars through plant breeding of modern domesticated tomato (*S. lycopersicum*), enabling production of diverse shapes, colors, and sizes of tomato (Bai et al., 2007).

Tomato is the second most widely cultivated vegetable crop across the world, behind potato (McGovern et al., 2015) with estimated yields of 170 million tons in 2014 and continued expansion (Guan et al., 2018). Tomatoes are considered "high value" both nutritionally and economically, with the major producing countries being China (30.7% of global production), India (11.5%), and the United States (8.1%), as of 2019 (Maurya et al., 2019). The use of greenhouse and other protected culture systems often allows for extended growing seasons, maximizing yields in top producing countries (Kalloo et al., 1991). In the United States, California and Florida are top producing states, with a growing season of October through June. In the summer months, domestic open-field tomato production primarily comes from Georgia, North Carolina, Ohio, Tennessee, and Virginia in the southeastern U.S. (Guan et al., 2018).

While tomatoes are high in demand and value, several factors have limited production and caused management adaptations, which has potentially reduced economic gains for tomato farmers. In Florida, tomato production decreased from 45,000 to 29,000 acres between the years 2001 to 2012 in response to changing soil fumigation practices and market dynamics related to tomato imports. The overall value of Florida tomato farms dropped tremendously from $620 million in 2010 to $270 million in 2017, and further declined to $260 million in 2019 (Cao et al., 2018) with estimated production declines of 10-25% per county (VanSickle et al., 2000). Previously, methyl bromide soil fumigation applications were regularly implemented, as it was an effective management practice for plant-parasitic nematodes, weeds, and soil-borne plant pathogens (Osteen, 2000) such as *Fusarium oxysporum* and *Ralstonia solanacearum* (Xie et al., 2015). Methyl bromide was a highly effective fumigant for soilborne pests, with tomato production accounting for 30% of all methyl bromide applications (Osteen et al., 2000). However, in 1992 methyl bromide was officially listed as a stratospheric ozone-depleting substance under the Montreal Protocol (Saltzman et al., 2022). This international treaty resulted

in the phase out of methyl bromide use in all countries by 2005, except for approved critical uses. While this change has been best for maintenance of the stratospheric ozone layer (Saltzman et al., 2022) the switch to alternative fumigants has contributed to 20% yield losses compared to methyl bromide treatments for Florida producers (Cao et al., 2018). This shift in production management practices, in combination with increased and changing pest and disease pressures due to climate change, results in a need for increased understanding of agroecological relationships with tomato to aid in alternative management practices to benefit producers while reducing environmental impacts.

Tomato Domestication

It is believed that the modern tomato has been domesticated from the wild ancestors of *S. pimpinellifolium* and *S. lycopersicum var. cerasiforme* (Jaiswal et al., 2020). The wide genetic diversity of wild tomato has allowed for extensive breeding of new tomato cultivars (Kalloo et al., 1991). In order to create desirable market products, tomato has undergone extensive selective breeding to obtain desirable production and marketing traits (Milla et al., 2017). Tomato types can be categorized along a domestication gradient from wild relatives, first domestication, second domestication, heirloom/landrace, and lastly to modern hybrid types (Jaiswal, et al., 2020). In comparison to the wild types, modern tomatoes have varying fruit sizes, shapes, and flavor profiles, larger seeds for replanting, greater root systems with smaller plant leaf tissues, and are often selected for adaptations to local environments (Bai et al., 2007; Jaiswal et al., 2020). Tomato breeding has been rather successful due to the relatively short life cycle, high fertility rate, distinct morphological characteristics, high self-fertility, and great reproductive potential (Kalloo et al. 1991). While the process of domestication has resulted in manipulation of plant morphological characteristics to allow for better management, increased yields, and improved culinary qualities, a result of this selective breeding is also what has been termed "domestication syndrome" as current tomato cultivars have lost 95% of their natural traits (Bergougnoux et al., 2013). This has resulted in a "bottle-necked" effect on genetics from wild species (Jaiswal et al., 2020) as breeders have selected naturally occurring recombinations to produce desired cultivars while rarely re-introducing genes from the wild germplasm (Bai et al., 2007). The loss of natural genetics from wild relatives to cultivated plants can have a direct impact on plant-microbe interactions and nutrient use efficiency, and these changes can also influence soil environments (Banani et al., 2013; Cordova-Campos et al., 2012). Reduced genetic diversity has potentially impacted tomato resiliency, the ability to create symbiotic relationships with beneficial fungi, and potentially decreased defense mechanisms against environmental stressors, such as soil-borne pathogens (Smulders et al., 2020; Jaiswal et al., 2020; Cordova-Campos et al., 2012; Banani et al., 2014).

Fusarium wilt

Tomato plants are hosts of more than 200 pests and diseases caused by pathogenic fungi, bacteria, viruses and nematodes (Jaiswal et al., 2020). *Fusarium* species are responsible for economically damaging plant diseases across a wide range of crops across the globe

(Duyvesteijn et al., 2005). *Fusaroum oxysporum* has 106 *formae speciales* (Edel-Hermann and Lecomte et al., 2019), which are informal taxonomic groupings of a parasite adapted to a specific host. Even though *Fusarium* has some host specificity, showing increased disease within prime hosts, an ability to infect a broad host range allows for increased survival and resilience (Mcgovern et al., 2015). Within the *formae speciales*, there are also different races, which are identified mutations that developed through fungal adaptation of *Fusarium*. In the case of tomato, *F. oxysporum* f. sp. *lycopersici (Fol)* has recognized races of 1, 2, and 3, which are all responsible for damping-off of tomato seedlings, basal stem necrosis, as well as yellowing of foliage and wilting and death of mature plants. *Fol* is characterized as a wilt disease, as it will penetrate tomato roots, spreading through the vasculature as hyphae clog xylem cells, preventing water-flow through the plant (Duyvesteijn et al., 2005). Symptoms in mature tomatoes are most noticeable after flowering and fruit set, often distinguishable by a one-sided appearance of wilt yellowing/browning of foliage which is enhanced by warm temperatures (~28° C) (Mcgovern et al., 2015).

While tomato production systems are variable and include both indoor and outdoor environments throughout temperate, subtropical and tropical regions, soilborne fungal pathogens such as *F. oxysporum* are a prevalent limiting factor in many environments (Kalloo et al., 1991; Khan and Khan, 2002; Duffy and Défago, 1999). *Fol* produces a variety of asexual reproductive structures, such as microspores, macrosptores, and chlamydospores. The chlamydospores enable long-term survival as their modified thick cell walls increase resiliency to stress factors such as nutrient depletion and extreme temperatures (Mcgovern et al., 2015; Michielse and Rep, 2009). The mycelia of *F. oxysporum* are also resilient and capable of surviving in alternative hosts and, in association with plant debris as saprophytes, and over winter within soils (Mcgovern et al., 2015; Michielse and Rep, 2009). The survival of *Fol* in the field can be up to 10 years if not fumigated (Mcgovern et al., 2015). Macro- and microconidia act as the asexual, infectious structures that allow for aerial sporulation for pathogen spread (Mcgovern et al., 2015; Michielse and Rep, 2009). These adaptations allow ease of transmission by wind, tools, insect vectors, and other factors. As *Fol* is extremely resilient in many environmental conditions and hosts, management practices of cropping systems can continue to enhance pathogen spread and severity through the addition of ammonium-based fertilizers and low soil pH. This has made *F. oxysporum* one of the most widely studied fungal plant pathogens (Mcgovern et al., 2015; Yergeau et al., 2006). Commercial fungicides such as benomyl, captafol, and imazalil are inconsistent and recommended concentrations often have phytotoxic effects to crops (Duffy and Défago, 1999). As there are still no recommended fungicides that can eradicate *Fusarium* diseases, use of preventative practices such as soil disinfestation and the development of *Fusarium* resistant plant cultivars have been beneficial to growers. However, there are limitations to the use of soil disinfestation on a mass plant production scale, and development of plant resistant cultivars is a lengthy process which is often only effective towards certain species/races of *Fusarium*. The resiliency of this pathogen in combination with the lack of

effective controls makes development of management strategies of high concern (Shrestha et al., 2021, Yergeau et al., 2006).

***Trichoderma* spp.**

While some soil microorganisms can be pathogenic, others are appreciated for their crucial roles in ecological functions (Asghar and Kataoka, 2021; Bader et al., 2019). For example, there are various plant growth-promoting microbes (PGPM) that are widely recognized for their ability to promote plant growth and productivity. *Trichoderma* is a fungal genus of opportunistic, free-living fungi that are widely distributed in global soils (Harman et al., 2004; Cai and Chen, 2014; Hoyos-Carvajal et al., 2009). This genus is characterized by rapid growth and resilient growth under a wide range of environmental conditions, such as varying nutrient availability, pH, and temperature. Competitive growth of *Trichoderma*, combined with its numerous benefits to plant production, make *Trichoderma* a genus of high interest and economic importance (Nawrocka et al., 2013; Bader et al., 2019; Asghar and Kataoka, 2021; Hoyos-Carvajal et al., 2009). Many *Trichoderma* spp. can colonize the roots and shoots of plants, such as tomato, and various strains have mechanisms with differing potential to improve plant growth and resiliency to biotic and abiotic stresses (Harman et al., 2004).

While the anamorph *Trichoderma* reproduces asexually, the associated teleomorph genus *Hypocrea* reproduces sexually as teleomorphs, which is common for isolates that colonize woody and herbaceous plant materials (Harman et al., 2004). Most *Trichoderma* spp. have evolved as avirulent, opportunistic plant symbionts, and will penetrate the first/second layers of plant roots with large variability in the degree of rhizosphere competence (the ability to colonize and grow in association with plant roots) among species/strains (Harman et al., 2004). Some are only able to colonize local sites of plant roots, while those possessing extensive rhizosphere competence can colonize entire root surfaces for weeks or months (Harman et al., 2004; Tucci et al., 2011). Plant roots colonized by effective *Trichoderma* isolates follow a mechanism known as "priming," which results in plant roots being "sensitized" to pathogen presence, through the induction of systematic plant resistance (Tucci, 2011; Hoyos-Carvajal et al., 2009; Chowdappa et al., 2013). Systematic resistance is the triggering of plant defenses by an outside stimulus (Mcgovern et al., 2015; Chowdappa et al., 2013). Induced resistance in plants are complex but can be generalized by two common defense mechanism responses; the "attack" of plant tissues will result in production of pathogenesis-related proteins within signal transduction pathways, as jasmonic acid is produced for herbivory predation while salicylic acid is produced for pathogenic micro-organisms (Harman et al., 2004; Tucci et al., 2011). The ability to induce systematic resistance, in combination with the ability of *Trichoderma* spp. to parasitize plant pathogens make these fungi of interest for biocontrol applications in cropping systems (Tucci et al., 2011; Hoyos-Carvajal et al., 2009; Chowdappa et al., 2013).

Trichoderma can parasitize soil fungi and nematodes, such as *Fol*, by attaching and coiling to the host surface where it will form appressoria (enlarged hyphal tips that penetrate cell

walls) and produce fungitoxic cell-wall degrading enzymes and antibiotics (Harman et al., 2004). This acts to inhibit or degrade plant-pathogen enzymes, lessening their ability to cause disease, and some strains having the capacity to produce over 100 antibiotic compounds (Harman et al., 2004). This works in tandem with increased competition for resources in soil and the crop rhizosphere, which also can limit the growth of pathogens and their severity in host plants, such as tomato. (Tucci et al., 2011; Hoyos-Carvajal et al., 2009; Chowdappa et al., 2013).

Studies using native *Trichoderma* isolates in various geographic areas have shown that *Trichoderma* act as antagonists to soil-fungal pathogens, including *Fol* in tomato, while also increasing plant growth. In Egypt, seven *Trichoderma* isolates were evaluated for biocontrol effects of *Fol* in tomato (Sallam et al., 2019). Results showed significant differences in antagonism among different *Trichoderma* species/strains, with the lowest disease severity resulting in only 24.8% infection of tomato plants (Sallam et al., 2019). In Iran, twenty-eight native *Trichoderma* isolates were analyzed for antagonism against *Fol* in dual culture lab assays and under greenhouse conditions with tomato (Barari et al., 2016). In dual culture, all *Trichoderma* exhibited *Fol* inhibition, which was measured by zones of inhibition among plate samples. This is a direct effect of *Trichoderma* enzymes, which limit growth of fungal pathogens. Within tomato pots, the application of *T. harzianum* (isolate N-8) resulted in d the greatest biocontrol potential with tomato disease severity of only 14.75%, along with the greatest plant height and dry weight compared to other treatment groups (Barari et al., 2016). Similarly, a study in Argentina assessed nineteen strains of *T. harzianum*, all of which exhibited 20-100% *Fol* suppression in dual culture assays (Bader et al., 2019). The top four biocontrol strains (FCCT16, FCCT58, FCCT 199-2, FCCT363-2) were then applied to tomato for *Fol* suppression. Tomato treatment groups displayed increased growth of 50-135% and increased shoot dry weights of 166-400% compared to controls. *Trichoderma* treatments also had a decrease in *Fol* disease severity from 10-30% compared to control groups (Bader et al., 2019).

Studies show that there is a direct correlation between plant domestication, resiliency, and soil-fungi interactions. Banani et al., (2014) concluded that efficacy of *Trichoderma* for resistance to downy mildew varied genetically between different *Vitis vinifera (*grape) cultivars grown in greenhouse settings. In the case of tomato, while analyzing the root-association of 27 genetically diverse tomatoes with soil bacterial communities, it was evident that bacterial functional groups involved in synthesis of certain amino acids or in carbohydrate metabolism were more often associated with wild relatives than modern varieties (Smulders et al., 2020).

Jaiswal et al., (2020) investigated the relationships between 25 genetically diverse tomato, beneficial *Trichoderma* strains, and the plant pathogens *Botrytis cinerea* (fungi) and *Phytophthora* (oomycete). The authors concluded that there were altered plant-microbe interactions based on tomato domestication, with wild relatives of tomato having greater resistance to *B. cinerea* compared to *S. pimpinellifolium*, *S. cerasiforme* (landrace/heirloom) and modern cultivars. Plant response benefits with *Trichoderma* also decreased along the domestication gradient, with wild relatives and early domestication being more responsive to

Trichoderma-induced benefits for increased shoot biomass and suppression of pathogens (Jaiswal et al., 2020). While there is supported evidence of the impacts of plant-genetics with soil-microbes, it is also important to consider how abiotic factors, such as soil nutrients, can impact these relationships (Cordova-Campos et al., 2012).

Inoculation of various *Trichoderma* spp. resulted in positive tomato growth and disease resistance compared to non-inoculated controls (Sallam et al., 2019; Barari et al., 2016; Bader et al., 2019). However, the effectiveness of induced resistance can vary between *Trichoderma* spp. in relation to various crop species/genotypes and pathogens (Table 1.1) (Harman et al., 2004). While many biological controls offer potential for improved pest management, a greater understanding of the ecological relationships between *F. oxysporum*, *Trichoderma*, and tomato germplasm is necessary to ensure implementation of the most effective management strategies (Khan and Khan, 2002; Yergeau et al. 2006).

Effects of Soil Phosphorus

Tomato crops, due to the high production of biomass, use high rates of soil nutrients. This significantly adds to production costs, and the high rates of inputs are associated with high energy use and potential environmental degradation when nutrients are lost to surface and groundwaters. Phosphorus (P), a finite global resource, can often limit crop productivity due to low solubility in soil and relatively high plant uptake. Phosphorus is one of seventeen essential nutrients required for plant growth, and the second most important macronutrient behind nitrogen (Balemi and Negisho, 2012). Phosphorus critical for plant growth and various functions, including photosynthesis, respiration (Balemi and Negisho, 2012). Over 40% of global soils are considered P-deficient (Balemi and Negisho, 2012). In the southeastern USA and other regions with highly-weathered soils (e.g., ultisols and oxisols in the USDA classification system), P availability is often limited due to the predominant highly weathered and acidic soils where P adsorbs strongly to clay minerals or iron (Fe) and aluminum (Al) oxides or forms secondary compounds with Fe or Al (Rinu et al., 2013; Bader et al., 2019).

Along with soil limitations, the potential of P uptake can also differ between different crop species/genotypes due to differing mechanisms (e.g., root morphological changes, root exudation to alter soil chemical and biological processes in the rhizosphere, associations with endophytic or associative microbes) that determine a plant's ability to uptake P or grow in areas where P is limited (Richardson et al., 2009; Balemi and Negisho, 2012). In the case of tomato, there are differences in tomato growth among genetically different varieties, e.g., Dixon et al., (2020) reported a 77% increase in tomato dry weight within P efficient tomato groups when grown under limited P conditions. This study concluded that potential strategies of tomato for increased P use efficiency included rapid P sensing, optimized root growth, and microbial synthesis. To encourage plant P use efficiency, a better understanding of how plant breeding and domestication impact plant P uses through plant morphology, metabolism, and ability to form relationships with beneficial microbes for P solubilization is needed (Balemi and Negisho, 2012; Hoyos-Carvajal et al., 2009; Dixon et al., 2020). Microorganisms are known to play a large role in the

biogeochemical cycling of P in agroecosystems, as the act of farming can result in transportation and translocation of P in soil and surrounding water systems. Some microorganisms have been reported to increase P availability for plants. Fungal species are considered more appropriate for nutrient management strategies, with types of phosphate-solubilizing fungi (PSF) being of greater use in agricultural systems as compared to bacteria due to larger occupancy range and greater resiliency within soil environments (Rinu et al., 2013; Doilom et al., 2020; Bononi et al., 2020). PSF have the potential to enhance the solubilization of phosphorus compounds by producing organic acids and mobilizing phosphorus compounds, such as those in P fertilizers and soil minerals, and to increase nutrient uptake and crop P availability (Doilom et al., 2020). This is because organic acids can convert soil P into mono or di-basic phosphates that are readily available for plant uptake (Bononi et al., 2020). However, P solubilization potential by PSF is heavily influenced by site-specific soil chemical conditions, i.e., the forms of phosphorus compounds and soil pH (Ngo et al., 2021; Elias et al., 2016).

Arbuscular mycorrhizal fungi (AMF) can form symbiotic relationships with over 70% of terrestrial plants, including most agricultural crops. They are recognized for their ability to enhance soil fertility, soil biodiversity, and nutrient cycling for plants, especially soil P. However, AMF benefits of nutrient acquisition are due to extensive growth of fungal hyphae networks throughout the soil microbiome, which is highly dependent on soil P conditions, as high levels can negativity impact mycorrhizal growth and productivity (Ngo et al., 2021).

In contrast to AMF, which rely heavily on plant symbiosis and soil microbiome conditions, *Trichoderma* is another PSF that can be beneficial in some agricultural environments due to its resiliency within a large range of environments. A previous study from Bononi et al., (2020) analyzed the relationship between *Trichoderma*, soybean (sci name), and multiple phosphorus fertilizer sources to determine if the fungi can improve phosphorus efficiency of highly weathered, acidic and P-deficient soils of the Amazon. Results showed that two *Trichoderma* isolates (AMS 34.39 and AMS 31.15) improved P solubilizing enzyme activity by 16-17% with rock phosphate and 10-15% with triple superphosphate. Enzymatic activities were greatest for rock phosphate due to its lower availability, resulting in greater microbial activities for mineralization. Phosphorus sources were compared at four different application levels, with the greatest increases of soybean biomass being at 70 kg P/ha, with other significant differences in plant height at P rates of 50, 70, and 90 kg P/ha with *Trichoderma*. While results showed differences within treatments, the combination of P and *Trichoderma* were able to increase plant growth and P availability in comparison to treatments without the fungi (Bononi, et al., 2020). In a similar study, different strains of *T. harzianum* and *T. asperellum* solubilized phosphoric rock or calcium phosphate for *Phaseolus vulgaris* (bean) production. They reported three possible mechanisms of P solubilization for bean growth including acidification and redox activity, with production of chelating metabolites being the most common mechanism (Hoyos-Carvajal et al., 2009).

Table 1.1: Evidence and effectiveness of induced resistance in various crops by Trichoderma spp. (adapted from Harman et al., 2004, reproduced in adapted form with permission from Springer Nature).

***Trichoderma* spp.**	**Crop**	**Pathogen**	***Trichoderma* effects**	**Time post application**	**Efficacy**
***T. virens* G-6, G-6-5 and G-11**	*Gossypium herbaceum*	*Rhizoctonia solani*	Plant protection	4 days	78% disease reduction
***T. harzianum* T-39**	*Phaseolus vulgaris*	*Colletotrichum lidemuthianum*; *botrytis cinerea*	Plant protection (leaves) with root inoculation of T-39	10 days	42% reduction in symptomatic spread on leaf tissues
***T. harzianum* T-39**	*S. lycopersicum*, *Capsicum annuum*, *Nicotiana tabacum*, *Phaseolus vulgaris*, *Lactuca sativa*	*Botrytis cinerea*	Plant protection (leaves) with root inoculation of T-39	7 days	25-100% symptomatic reduction
***T. asperellum* T-203**	*Cucumis sativus*	*Pseudomonas syringae pv. lachrymans*	Plant protection (leaves) with root inoculation of T-203	5 days	80-100% symptomatic reduction on leaf tissues and of bacterial cells
***T. harzianum* T-22**	*S. lycopersicum*	*Alternaria solani*	Plant protection with root inoculation of T-22	3 months	Up to 80% symptomatic reduction

As studies show, some *Trichoderma* can increase nutrient availability through mineralization and solubilization of limiting elements, such as P, within the rhizosphere (Cai and Chen, 2014). Evidence also supports the ability of *Trichoderma* to parasitize pathogenic fungi through production of antibiotic compounds, and colonization of plant roots to increase root structure and overall plant growth (Harman, 2004; Bader et al., 2019). As varying forms of soil P can impact growth, as well as interactions of plant growth and *Trichoderma*, evidence also supports that P availability can influence disease resistance of plants, namely when the ability of a host plant controls the severity of infection when environmental conditions favor the pathogen (Mcgovern et al., 2015). Studies show that *Fusarium* severity is slightly increased with P fertilization, but not as drastically as with different forms of N fertilization, while the combination of N and P fertilization has high disease severity in some tomato plants (Veresoglou et al., 2012). It is also important to consider how tomato domestication and plant genotypic diversity can influence nutrient-use efficiency (Dixon et al., 2020). There is still limited information regarding the relationships of *Trichoderma* and P uptake in highly weathered, acidic soils for organic production. This in combination with limited information of how fertilization and nutrient availability by PSF, such as *Trichoderma*, can impact the severity of disease caused by soil-borne pathogens, such as *F. oxysporum*, and the influences of the rhizosphere– plant interactions among tomato germplasm calls for ongoing investigation (Srihuttagum et al., 1989).

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CHAPTER TWO

NATIVE *TRICHODERMA* ISOLATES VARY IN BIOCONTROL ACTIVITY AGAINST TOMATO WILT CAUSED BY *FUSARIUM OXYSPORUM* AND IN PHOSPHORUS SOLUBILIZATION POTENTIAL

Abstract

Beneficial soil fungi, such as *Trichoderma* spp., are known to improve crop growth, stress tolerance, and disease resistance. Many *Trichoderma* isolates can parasitize structures of plant pathogenic fungi, solubilize poorly-soluble soil nutrients such as phosphorus (P), and induce crop resistance to plant pathogens. However, little information exists on how *Trichoderma*-induced benefits to crops interact (i.e., nutrient uptake and disease tolerance) or the magnitude of benefits to crop production. In two objectives, we evaluated eight native *Trichoderma* isolates (including *T. afroharzianum, T. arundinaceum, T. asperelloides, T. asperellum, T. hamatum, and* multiple isolates in the *T. harzianum* species complex). In objective 1, eight *Trichoderm*a isolates were analyzed for potential to reduce *Fusarium wilt* caused by *Fusarium oxysporum* f. sp. *lycopersici (Fol)* in tomato (*Solanum lycopersicum* cv. *Valencia*) under greenhouse pot conditions. Treatments included *Trichoderma* (T1 to T8) with *Fol* and two control groups (1: no *Trichoderma*/no *Fol*, 2: no *Trichoderma*/with *Fol*). *Trichoderma* treatments increased biomass and reduced disease symptoms compared to the *Fol* inoculated control, with isolates T3 and T5 showing the greatest potential. In objective 2, culture assays of *Trichoderma* were conducted for 1) potential to solubilize poorly soluble secondary P compounds (aluminum (Al-P) or calcium phosphate (Ca-P)) based on fungal growth and P concentration in Pikovskaya's (PVK) broth, and 2) competitive interactions with *Fol* in dual culture assays on PVK agar. The PVK media was prepared with Al-P, Ca-P, and controls of highly available potassium phosphate (K-P) or no added P (No-P). In broth assays, mycelial biomass of *Trichoderma* and broth P concentrations were analyzed. Isolate T8 had the highest biomass in Al-P and Ca-P compared to other isolates/P sources. In dual culture, isolates were analyzed for competition, antagonism, and *Trichoderma* colony diameter to *Fol* colony diameter ratios were calculated to compare differences between isolates among treatment groups. *Trichoderma* isolates T5, T6, T7, and T8 had larger colony diameter ratios when grown on Al-P. Our results indicate that soil chemical environment and *Trichoderma* isolate nutrient acquisition traits may play an important role in biocontrol outcomes.

Introduction

Tomato (*Solanum lycopersicum*) is the second most widely cultivated vegetable crop globally, behind *Solanum tuberosum* (potato) (McGovern et al., 2015) with estimated yields of 170 million tons in 2014 and continued expansion (Guan et al., 2018). While tomatoes are high in demand and value, there are many factors that have limited production and caused management adaptations, and potentially reduced economic gains for tomato farmers. *Fusarium* species are responsible for economically damaging plant diseases across a wide range of crops across the globe (Duyvesteijn et al., 2005). In the case of tomato, *F. oxysporum* f. sp. *lycopersici (Fol)* can lead to damping-off of tomato seedlings, basal stem necrosis, as well as yellowing of foliage and wilting and death of mature plants. *Fol* is characterized as a wilt disease, as it will penetrate tomato roots, spreading through the vasculature as hyphae clog xylem cells, preventing water-flow through the plant (Duyvesteijn et al., 2005.) The resiliency of this pathogen in

combination with the lack of effective controls make development of management strategies of high concern (Shrestha et al., 2021, Yergeau et al., 2006).

While some soil microorganisms can be pathogenic, others are appreciated for their crucial roles in ecological functions (Asghar and Kataoka, 2021; Bader et al., 2019). For example, there are various plant growth-promoting microbes (PGPM) that are widely recognized for their ability to promote plant growth and productivity. *Trichoderma* is a fungal genus of opportunistic, free-living fungi that are widely distributed in global soils (Harman et al., 2004; Cai and Chen, 2014; Hoyos-Carvajal et al., 2009). This fungal genus is characterized by its rapid growth and resiliency to grow under a wide range of environmental conditions including those of varying nutrient availability, pH, and temperature. This, in combination with its numerous benefits to plant production, makes *Trichoderma* a species of high interest and economic importance (Nawrocka et al., 2013; Bader et al., 2019; Asghar and Kataoka, 2021; Hoyos-Carvajal et al., 2009). As many *Trichoderma* spp. can colonize the roots and shoots of plants, such as tomato, various strains have mechanisms with differing potentials to improve plant growth and resiliency to biotic and abiotic stresses (Harman et al., 2004). Many *Trichoderma* species can also parasitize pathogenic soil fungi, such as *Fol*, by attaching and coiling itself to the host surface where it will form appressoria (enlarged hyphal tips that penetrate cell walls) and produce fungitoxic cell-wall degrading enzymes and antibiotics (Harman et al., 2004). This acts to inhibit or degrade plant-pathogen enzymes, lessening their ability to cause disease, with some strains having the capacity to produce over 100 antibiotic compounds (Harman et al., 2004). This trait, in combination with increased competition for resources in soil and the crop rhizosphere, can limit the growth of pathogens and their severity in host plants, such as tomato. (Tucci et al., 2011; Hoyos-Carvajal et al., 2009; Chowdappa et al., 2013).

Studies using different native *Trichoderma* isolates in various geographic areas have demonstrated that *Trichoderma* can act as an antagonist to soil-fungal pathogens, including *Fol* in tomato, while also increasing plant growth. In Egypt, a study evaluated seven *Trichoderma* isolates for biocontrol effects of *Fol* in tomato (Sallam et al., 2019). Results showed significant differences, supporting ideas that *Trichoderma* antagonism varies among species/strains, with the lowest disease severity resulting in only 24.8% infection of tomato plants (Sallam et al., 2019). In Iran, twenty-eight native *Trichoderma* were analyzed for antagonistic effects on *Fol* in dual culture lab assays and under greenhouse conditions with tomato (Barari et al., 2016). Within dual culture, all *Trichoderma* exhibited *Fol* inhibition, which was measured by size of a zone of inhibition among plate samples. This is a direct effect of *Trichoderma* enzymes that limit growth of fungal pathogens. Within tomato pots, the application of *T. harzianum* (N-8) exhibited the greatest biocontrol potential with tomato disease severity of only 14.75%, along with the greatest plant height and dry weight compared to other treatment groups (Barari et al., 2016).

Tomato crops, due to the high production of biomass, use high rates of soil nutrients. This significantly adds to production costs, and the high rates of inputs are associated with high energy use and potential environmental degradation when nutrients are lost to surface and

groundwater. Phosphorus (P), a finite global resource, can often limit crop productivity due to low solubility in soil. Over 40% of global soils are considered P-deficient (Balemi and Negisho, 2012). In the southeastern USA and other regions with highly-weathered soils (e.g., Ultisols and Oxisols in the USDA classification system), P availability is often limited due to the predominant highly weathered and acidic soils where P absorbs strongly to clay minerals or iron (Fe) and aluminum (Al) oxides, or forms secondary compounds with Al or Fe (Rinu et al., 2013; Bader et al., 2019).

Microorganisms are known to play a large role in the biogeochemical cycling of P in agroecosystems, as some have been reported to increase P availability for plants. Fungal species are considered more appropriate for nutrient management strategies, with types of phosphate-solubilizing fungi (PSF) being of greater use in agricultural systems as compared to bacteria due to larger occupancy range and greater resiliency within soil environments (Rinu et al., 2013; Doilom et al., 2020; Bononi et al., 2020). PSF have the potential to enhance the solubilization of P compounds by producing organic acids and mobilizing P compounds, such as those in P fertilizers and soil minerals, and to increase nutrient uptake and crop P availability (Doilom et al., 2020). This is because organic acids can convert soil P into mono or di-basic phosphates which are readily available for plant uptake and use (Bononi et al., 2020). However, P solubilization potential by PSF is heavily influenced by site-specific soil chemical conditions, in particular the forms of P compounds and soil pH (Ngo et al., 2021; Elias et al., 2016).

Trichoderma spp. are potential PSF that could be beneficial in some agricultural environments due to their resiliency within a large range of environments. A previous study analyzed the relationship between *Trichoderma*, *Glycine max* (soybean) and multiple P fertilizer sources to determine if the fungi can improve P efficiency of highly weathered, acidic and P deficient soils of the Amazon. Results showed that two *Trichoderma* isolates (AMS 34.39 and AMS 31.15) improved enzyme activity by 16-17% with rock phosphate and 10-15% with triple superphosphate. Enzymatic activities are greatest for rock phosphate due to its lower availability, resulting in greater microbial activities for mineralization. P sources were compared at four different application rates, with greatest increases of soybean biomass being at 70 kg/ha, with other significant differences in plant height at P rates of 50, 70, and 90 kg/ha with *Trichoderma*. While results showed differences within treatments, the combination of P and *Trichoderma* significantly increased plant growth and P availability in comparison to treatments without the fungi (Bononi et al., 2020). In a similar study, they observed different strains of *T. harzianum* and *T. asperellum* were able to solubilize phosphoric rock or calcium phosphate for bean production. They reported three possible mechanisms of P solubilization for bean growth including acidification and redox activity, with chelating of metabolites being the most common mechanism (Hoyos-Carvajal et al., 2009).

As studies have shown, some *Trichoderma* isolates can increase nutrient availability through mineralization and solubilization of limiting elements, such as P, within the rhizosphere (Cai and Chen, 2014). Evidence also supports the ability of *Trichoderma* to parasitize pathogenic

fungi through the production of antibiotic compounds and colonize plant roots to increase root structure and overall plant growth (Harman et al., 2004; Bader et al., 2019). As varying forms of soil P can impact growth and interactions of plant growth and *Trichoderma*, evidence also supports that fertilization can influence disease resistance of plants, i.e., the ability of a host plant to control the severity of infection when environmental conditions favor the pathogen (Mcgovern et al., 2015). Studies show that *Fusarium* severity is slightly increased with P fertilization, but not as drastically as N fertilization, while the combination of N and P fertilization had the highest disease severity in some tomato plants (Veresoglou et al., 2012). It is also important to consider how tomato domestication and plant genotypic diversity can influence nutrient-use efficiency (Dixon et al., 2020). There is still limited information regarding the relationships of *Trichoderma* and P uptake in highly weathered, acidic soils for organic production, and limited information of how fertilization and nutrient availability by PSF, such as *Trichoderma*, can impact the severity of disease caused by soil-borne pathogens, such as *Fol*, and other influences in the rhizosphere (Srihuttagum et al., 1989).

To better understand how native *Trichoderma* isolates play a role in suppression of *Fusarium* and soil P availability, our objectives were to evaluate evaluate eight native *Trichoderma* isolates for 1) suppression of tomato wilt disease caused by *Fol* in a susceptible heirloom tomato cultivar, and for 2) solubilization of Al-phosphate and Ca- phosphate in vitro assays, and to analyze interactions with *Fol* in dual-culture plate assays on media with varying P substrates. We hypothesized that 1) the ability of *Trichoderma* isolates to reduce symptoms of *Fol* on cv. Valencia tomato compared to a *Fol*-inoculated control would vary among isolates, with some native isolates being equivalent to a non-*Fol* inoculated control, and 2) *Trichoderma* isolates would vary in ability to solubilize P sources, with native isolates more effective in solubilization of Al- than Ca-phosphates, which would impact interactions with *Fol* in dual-culture plate assays.

Methodology

Objective 1. Biocontrol effects of eight *Trichoderma* isolates from Tennessee (Table 2.1) were evaluated in a randomized complete block greenhouse experiment in soil inoculated with *Fol.* Non-*Trichoderma* inoculated treatments (C1, no *Fol* inoculation and C2, with *Fol* inoculation) were included as controls. There were four replicates of each treatment group in tomato pots, and the study was conducted three times.

Trichoderma isolates (Table 2.1) and *Fol* were cultured on potato dextrose agar (PDA); 31.2 g / 800 mL sterile deionized water with 0.4 mg rifampicin in 85-mm Petri dishes. *Trichoderma* was grown for 5 to 7 days under continuous, fluorescent lighting at room temperature (~24°C) to allow for dense, conidial production, and *Fol* was grown under continuous, fluorescent lighting at room temperature (~22 °C) for 10 to 14 days to allow for dense, white hyphal growth to form and encourage sporulation (Jaiswal et al., 2020). Afterwards, *Fol* was cultured on organic red wheat *(Triticum aestivum)* seed. Wheat seed (150g) was soaked in deionized water for 24 hr, excess water drained, and the wheat separated into flasks of ~ 25 in each. Flasks were covered and

sterilized by autoclaving on slow settings for 25 min, which was repeated for 2 cycles within a 24 hr period. The autoclaved wheat seeds were inoculated with ten, 5 to 7 mm diameter plugs of *Fol* grown on PDA. Flasks were shaken and stored under supplemental fluorescent lighting at room temperature for 7 to 10 days until dense, white conidial growth was vident. *Fol* inoculum density was assessed with standard dilution plating to determine the mass of *Fol* inoculum needed for target application rates of 100,000 CFUs per g of soil (Shrestha et al., 2020).

To generate spore suspensions to inoculate tomato seeds, *Trichoderma* cultures were scraped (5 to 7 mm) with a sterile toothpick and deposited into a suspension using a stock solution of 0.1% Tween-twenty (Thermo-Fisher, Waltham, MA) in DI water to create a suspension of 25% Tween-twenty (Thermo-Fisher, Waltham, MA) stock to 75% DI water. The concentration of conidia in each solution was determined using a hemocytometer under a light microscope. Suspensions were diluted into a solution of 1% carboxy-methylcellulose and sterile water to achieve a final concentration of 1×10^6 spores/mL (Shrestha et al., 2020).

Tomato seeds of an heirloom cultivar (cv. *Valencia*) were surface sterilized with a 2 min soak in 3% hydrogen peroxide, followed by a rinse with sterile water, and the air-dried for 10 min in a biosafety cabinet. Seeds were soaked in each *Trichoderma* treatment (Table 2.1) conidial suspension for 15 min, while control seeds were soaked in 50% sterile water and 50% of an aqueous 1% carboxymethylcellulose solution. Treated seeds were planted in organic plant growth media (Lambert Organic All-Purpose Mix, Lambert, Québec, Canada) and grown in a greenhouse (25 °C average temperature) for 3 to 4 weeks in seedling trays prior to being transplanted. The number of live plants for each treatment was recorded in week 3.

In each trial, pots (10 cm diameter) were filled ¾-full with moistened organic plant growth medium. Pots for all *Trichoderma* treatments (isolates T1 to T8) and the non-*Trichoderma* inoculated C2 control were inoculated with *Fol* inoculum (100,000 CFU/g soil) by placing the inoculum at the surface of the 3/4-filled pot and covering it with a layer of plant growth medium to fill the pot. The C1 control was not inoculated with *Trichoderma* or *Fol*. *Trichoderma*-inoculated and non-inoculated control tomato seedlings were transplanted in respective treatment pots and grown for 9 weeks. At week 6 post-transplant, soil was allowed to dry between waterings to better observe tomato wilt induced by *Fol* infection. Wilt ratings during drought stress were recorded on a scale of 1 to 4 (1= 1 to 25%, 2= 26 to 50%, 3= 51 to 75%, and 4= 75 to 100%). Wilt ratings were based on visible turgor pressure and overall plant response to drought stressed conditions. Plant wilt stress ranged from 25% increments of those with no drought stress or visible wilting (rating of 1) to those that were completely wilted (rating of 4). Disease ratings followed a similar 1 to 4 rating scale based on 25% increments of above ground tissues turning yellow/brown, which is a visual symptom of *Fol* infection. Ratings were recorded once a week from weeks 6 to 9 (post-transplant) and 2 days after recovery from drought stress.

Table 2.1: Trichoderma isolates of interest for Objectives 1 and 2.

***Trichoderma* species**	**Project abbreviation**	**Strain name**	**GenBank Accession No.**	**Isolate origin**
T. harzianum species complex	T1	US10c	KY552264	*Agroathelia rolfsii* sclerotia, UT Organic Crops Unit
T. harzianum species complex	T2	OCURG	ON847372 (ITS14)/ON892783(EF12)	Soil, UT Organic Crops Unit
T. arundinaceum	T3	TBN	n/a	Soil, UT Organic Crops Unit
T. hamatum	T4	US10g	KY552263	*A. rolfsii* sclerotia, UT Organic Crops Unit
T. harzianum species complex	T5	OCURK	n/a	Soil, UT Organic Crops Unit
T. asperellum	T6	US	MH130213	*A. rolfsii* sclerotia, UT Organic Crops Unit
T. asperelloides	T7	OCUTK	n/a	Soil, UT Organic Crops Unit
T. afroharzianum	T8	SAP	ON892782	Soil, UT Plateau Research & Education Center
T. harzianum	T9	KRL-AG2	n/a	Koopert Biological Systems Inc.

At 9 weeks post-transplant, height (cm) and stem caliper (mm) were recorded. Tomato plants were removed from pots, cleaned, and roots separated from shoot tissue. Tomato shoot tissue was dried at 65 °C for 48 hours prior to recording dry biomass. Tomato root systems were assayed for colonization by *Trichoderma* spp. by assessing *Trichoderma* growth from surface-sterilized root pieces on semi-selective media. From each plant sample, fine tertiary roots were cut into 1-to-2 cm lengths and arranged in 10 evenly spaced clusters on *Trichoderma* selective media (TSM); 31.2 g potato dextrose agar, 0.15 g rose bengal, 0.3 g streptomycin, 0.3 g chloramphenicol per 800 mL sterile water (Askew and Laing, 1993; Williams et al., 2003). Photo documentation was collected after 3, 5, and 7 days of culture growth for quantitative assessment.

A mixed model factorial analysis of variance (ANOVA; Statistical Analysis Software, version 9.3) for treatment (*Trichoderma* isolates) and control (C1 and C2) effects on response variables was conducted. Data were evaluated for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) and data were transformed to address non-normal distribution and unequal variances of residual error where appropriate. "Trial" was treated as a random factor. Fisher's protected Least Significant Difference (P-LSD) test was used to separate means at 5% significance level; untransformed means and standard errors are reported. SAS/ANOVA was used to conduct mixed model analysis of variance for each data set to determine significance and normality.

Objective 2. To assess solubilization of aluminum (Al-P) and calcium (Ca-P) phosphates by *Trichoderma* isolates, a Pikovskaya's (PVK) broth assay was performed based on the methodology described by Doilom et al., (2020). Al-P and Ca-P treatments were compared to highly-available potassium phosphate (K-P) and no-phosphate (No-P) treatments which were control groups. First, 800 mL of PVK broth was prepared (0.4 g yeast extract, 8 g glucose, 0.40 g ammonium sulfate, 0.16 g potassium chloride, 0.08 g magnesium sulfate, and 0.0024 g ferrous sulfate per 800 mL sterile water) containing different phosphorus sources; aluminum phosphate ($AlPO_4$), 3.40 g; mono-potassium-phosphate (KH_2PO_4), 3.15 g; and tri-calcium phosphate [$Ca_3(PO_4)_2$], 4.00 g; and the No-P control treatment (Rinu et al., 2013; Doilom et al., 2020). Phosphate source rates were determined to provide equivalent rates of elemental P for each P source. Erlenmeyer flasks (125 mL) were sterilized and filled with 50 mL of PVK broth with three replicates for each P treatment with one of nine *Trichoderma* isolates (Table 2.1) for a total of 108 flasks. Plugs (5 mm diameter) of *Trichoderma* were added to each flask (Doilom et al., 2020; Rinu et al., 2013; Elias et al., 2016). Inoculated flasks were shaken at 130 rpm continuously for 7 days at room temperature with no light source. After 7 days, broth was poured through Whatman grade 2 filter paper into 50 mL centrifuge tubes to separate the mycelium. Broth was then centrifuged at 5000 rpm so that any mycelial fragments would settle to the bottom of the sample. Using a syringe filter (0.2-µm), 5 mL of broth samples were collected and stored at -37 °C prior to analysis for P concentration.

Filter papers with *Trichoderma* were left to air dry for 7 days under the biosafety cabinet and then weighed to record dry weight of mycelium. To analyze P concentration of broth samples, methods were modified from D'Angelo et al. (2001). In brief, 5 mL of samples were acidified with 5 mL of 0.0125 M H_2SO_4 + 0.05 M HCl solution and then reacted with ammonium molybdate tetrahydrate, followed by Malachite green carbinol hydrochloride in polyvinyl alcohol. The concentrations of inorganic P in broth solution at the end of incubation were determined by measuring absorbance at 630 nm and comparing absorbance to a standard curve.

The relative competitiveness of *Trichoderma* isolates with *Fol* was evaluated in a dual culture assay with variable P sources. Each *Trichoderma* isolate (Table 2.1) and *Fol* were cultured on PDA by methods described previously. For the dual culture, Pikovskaya's Agar Media (PVK agar; 0.40 g yeast extract, 8 g glucose, 12 g water agar, 0.40 g ammonium sulfate, 0.16 g potassium chloride, 0.08 g magnesium sulfate, 0.0024 g ferrous sulfate per 800 mL sterile water) was prepared containing different phosphorus sources as described previously for PVK broth ($AlPO_4$, 3.40 g; KH_2PO_4, 3.15 g; $Ca_3(PO_4)_2$), 4.00 g, and a no-P control). The PVK media plates (85 mm) were inoculated with a 5 mm plug of *Fol* on one side of the plate. After 3 days, a *Trichoderma* plug was added to the opposite side of the plate (Fig. 2.1), with three replicate plates for each *Trichoderma* isolate (T1 to T9). Plates were incubated for 7 days in a dark environment at room temperature. Fungal growth diameter was measured (mm) per colony/plate 7 days post *Trichoderm*a inoculation. These values were used to create average growth ratios of *Trichoderma* isolates to *Fol* within the dual culture plates. Competition ratings were collected and based on a 1 to 4 scale (1: 1 to 25%, 2: 26 to 50%, 3: 51 to 75%, 4: 76 to 100%; Fig. 2.1) and were also measured 7-days-post *Trichoderma* inoculation (Rinu et al., 2013; Doilom et al., 2020; Gazis et al., 2018). Colonies were assigned sporulation and culture density ratings using the same scale (1 to 4) and assessment methods to document differences in morphology (Fig. 2.2 and 2.3). Sporulation ratings were based on darkness of coloration while density ratings were based on the density of mycelial growth. Colonies were also assigned color codes/names from the Methuen Handbook of Colour (Wanscher et al., 1967) when grown on PDA compared to PVK with varying P sources in dual culture. This combination of ratings and color assignments allowed us to identify differences in growth patterns and isolate morphology.

Results

Objective 1. There were no significant effects of *Trichoderma* isolate inoculation on tomato seed emergence and establishment (data not shown). Dry tomato shoot biomass was affected by *Trichoderma* inoculation treatment ($P = 0.04$). The non-*Fol* inoculated control (C1; no *Trichoderma*/no *Fol*) had the highest biomass (2.5 g/plant), which was not different to the biomass for isolates T3 and T5 (~2.2 g/plant) with *Fol* inoculation (Fig. 2.3). The *Fol*-inoculated control (C2; no *Trichoderma*/with *Fol*) had the lowest average biomass (~1.7 g/pot). Plant biomass for all other *Trichoderma* isolate treatments were not different to the control treatment C2 (Fig. 2.3).

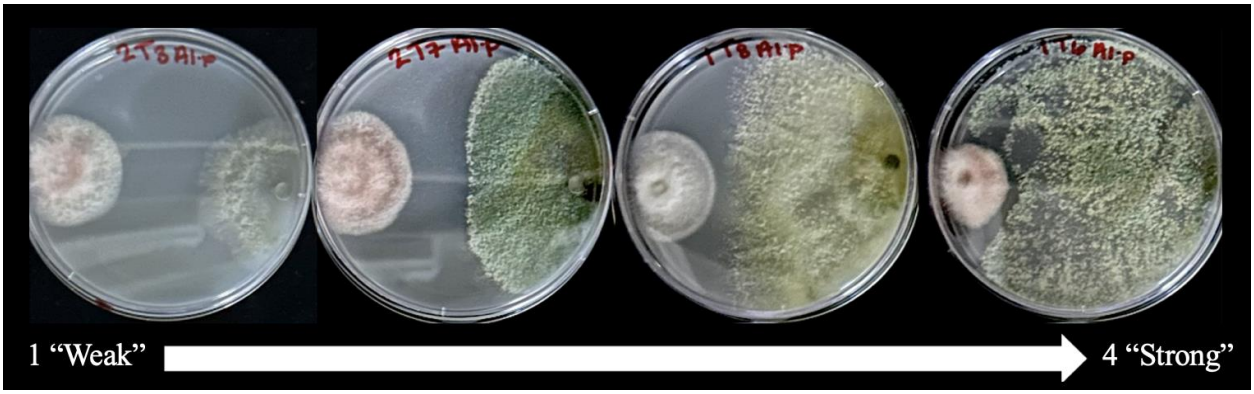

Figure 2.1: Examples of Trichoderma (right) and Fol (left) dual culture assay on PVK with Al-P to represent competition scale. Plates are in order from left to right from lowest (1 "weak") to highest (4 "strong") competition ratings. Ratings were based on a 1 to 4 scale (1: 1 to 25%, 2: 26 to 50%, 3: 51 to 75%, 4: 76 to 100%) of Trichoderma growth toward the Fol colony, measured 7-days-post Trichoderma inoculation (Gazis et al., 2018).

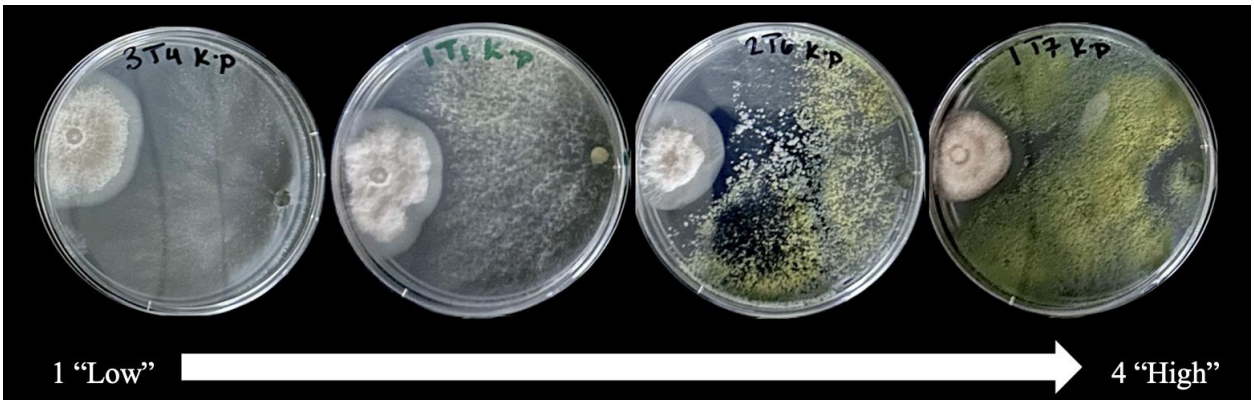

Figure 2.2: Examples of *Trichoderma* (right) and *Fol* (left) dual culture assay on PVK with K-P to represent sporulation and density scales. Plates are in order from left to right from lowest (1 "low") to highest (4 "high") growth ratings of *Trichoderma*. Ratings were based on a 1 to 4 scale (1: 1 to 25%, 2: 26 to 50%, 3: 51 to 75%, 4: 76 to 100%) of visible sporulation, measured 7-days-post *Trichoderma* inoculation (Gazis et al., 2018).

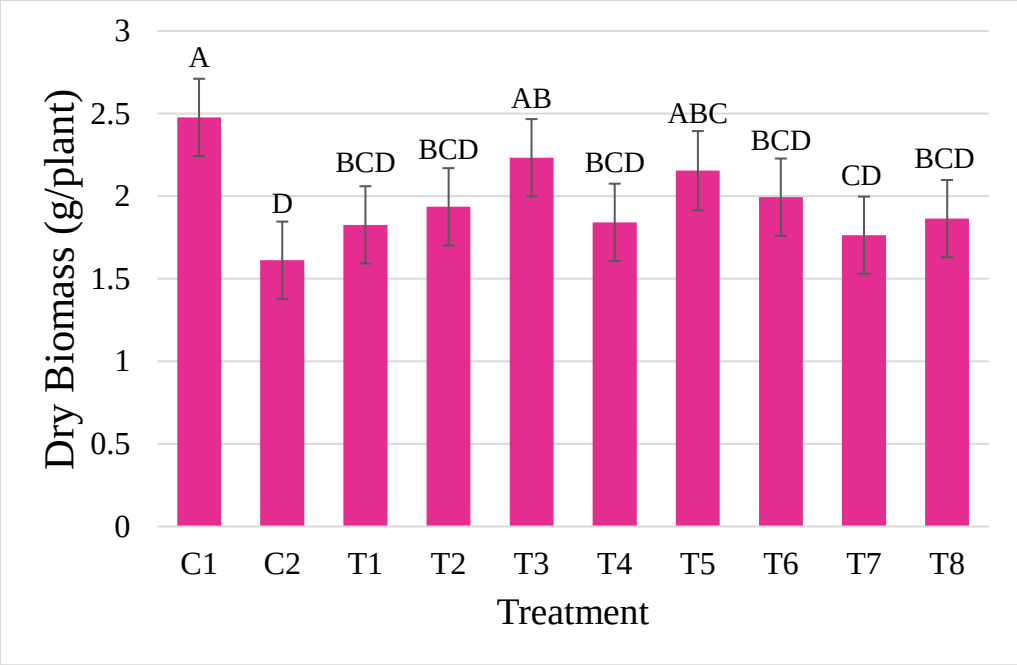

Figure 2.3: Mean tomato dry biomass (g/plant) among treatments for Objective 1 trials. *Trichoderma* treatment groups T1-T8 correspond to isolates listed in Table 2.1. Control treatments are represented by C1 (no *Trichoderma* and no *Fol*) and C2 (no *Trichoderma* with *Fol* inoculation). Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

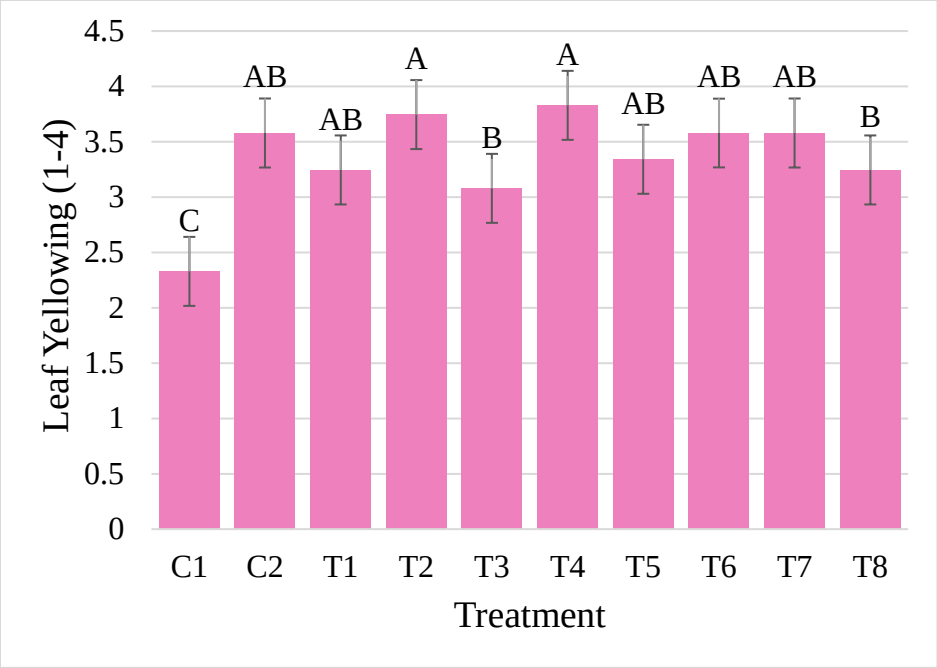

Figure 2.4: Mean tomato leaf tissue yellowing rating as affected by treatment in Objective 1 trials. *Trichoderma* treatment groups (T1 to T8) correspond to isolates listed in Table 2.1. Control treatments are represented by C1 (no *Trichoderma* and no *Fol*) and C2 (no *Trichoderma* with *Fol* inoculation). Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

Tomato plant ratings of leaf tissue yellowing as a measure of disease were significantly affected by *Trichoderma* and *Fol* inoculation (P=0.0005). Leaf yellowing was lowest in control treatment C1 (no *Trichoderma*/no *Fol*), intermediate for treatments T3 and T8, and highest in control treatment C2 (no *Trichoderma* with *Fol*) and *Trichoderma* treatments T2 and T4, which were not different than isolates T1, T5, T6, and T7 (Fig. 2.4). Tomato wilt during drought stress periods was similarly affected by *Trichoderma* and *Fol* inoculation (P=0.01). The lowest observed wilt ratings were in the control treatment C1 (no *Trichoderma*/no *Fol*) and *Trichoderma* treatments T1 and T8, which did not differ (Fig. 2.5). Wilt ratings of all other *Trichoderma* treatments did not differ from the *Fol*-inoculated control (C2).

Qualitative data for root colonization of *Trichoderma* and *Fol* was consistent between treatments that fungal colonies were present throughout the experiment, with some significant differences between colonization of *Fol* (P=0.01) and *Trichoderma* (P<0.0001). *Fol* colonization was significantly lowest (0.75%) in T3 tomatoes compared to other *Trichoderma* treatments. There was also evidence of colonization of both *Trichoderma* and *Fol* in control plants. To determine the impact of cross contamination, soil dilutions of control plants were analyzed to determine the colony forming units (CFU) present for both *Trichoderma* and *Fol*. The CFU were < 50/g of soil, compared to the application rates of 100,000 CFU/g of soil (*Fol*) and 1×10^6 spores/mL of *Trichoderma*. This analysis, in combination with root colonization rates in control plants being the lowest (0.27%) allows us to assume there were no significant microbial interferences to the results.

Objective 2. Concentrations of P in broth varied significantly among *Trichoderma* isolates ($P < 0.007$), P sources ($P < 0.0001$), and the interaction of these factors ($P = 0.02$). Among P-sources, K-P treatments had the highest P concentration (314 mg P/L) and were higher than Al-P (91.7 mg P/L) and Ca-P (102 mg P/L), which were statistically similar (Fig 2.6a). As expected, no P (0 mg P/L) was present in the No-P control treatment. In PVK broth with Al-P, isolates T8 (159 mg P/L) and T9 (154 mg P/L) had the highest concentrations, while T6 (22.0 mg P/L) had the lowest P concentration and T3 (42.7 mg P/L) the second lowest compared to other isolates (Fig 2.6b). Similarly, in PVK broth with Ca-P, T8 (146 mg P/L) had the highest P concentration while T6 (32.0 mg P/L) had the lowest concentration, and T2 (125 mg P/L) had the second lowest concentration P (Fig. 2.6c). In PVK broth with K-P, all isolates were statistically similar with the highest P concentrations compared to other P sources, with a range of 348 mg P/L to 283 mg P/L (Fig. 2.6d).

Average dry biomass of *Trichoderma* mycelium grown in PVK broth exhibited significant differences between phosphorus sources ($P < 0.0001$), *Trichoderma* treatment ($P < 0.0001$), and their interaction ($P < 0.0001$). Averaged across *Trichoderma* treatments, the highest overall fungal biomass in PVK broth was observed in Ca-P treatments (0.5851 g/flask), followed by Al-P treatments (0.4936 g/flask) (Fig. 2.7a). In contrast, K-P (0.3152 g/flask) was statistically similar to No-P treatments (0.3058 g/flask), which had the lowest mycelium biomass. In PVK broth with Al-P (Fig. 2.7b), isolates T1 (0.647 g/flask) and T8 (0.649 g/flask) had the highest

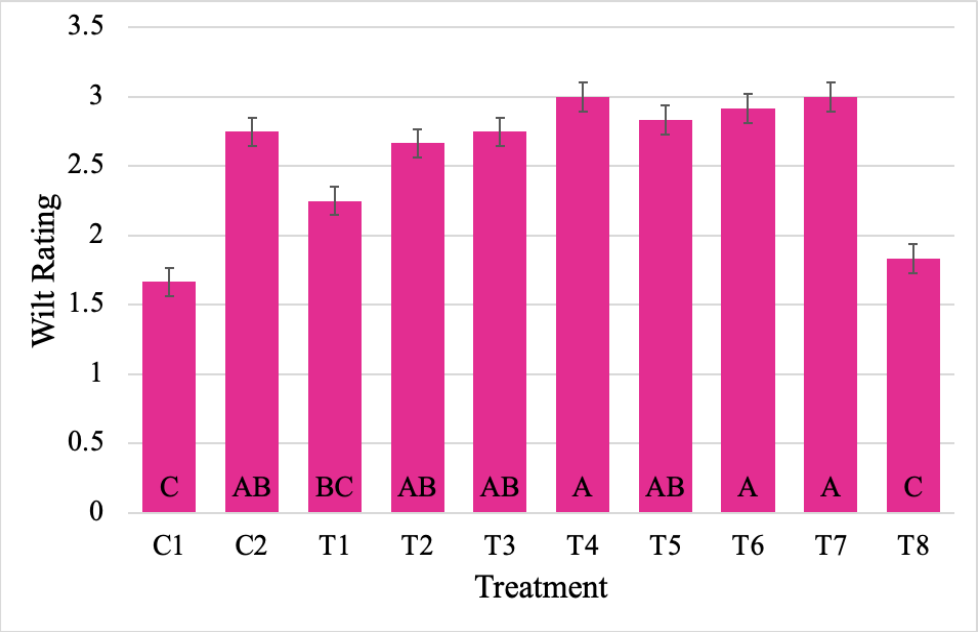

Figure 2.5: Mean tomato leaf wilt rating during drought stress periods as affected by treatment. *Trichoderma* treatment groups (T1 to T8) correspond to isolates listed in Table 2.1 for Objective 1 trials. Control treatments are represented by C1 (no *Trichoderma* and no *Fol*) and C2 (no *Trichoderma* with *Fol* inoculation). Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

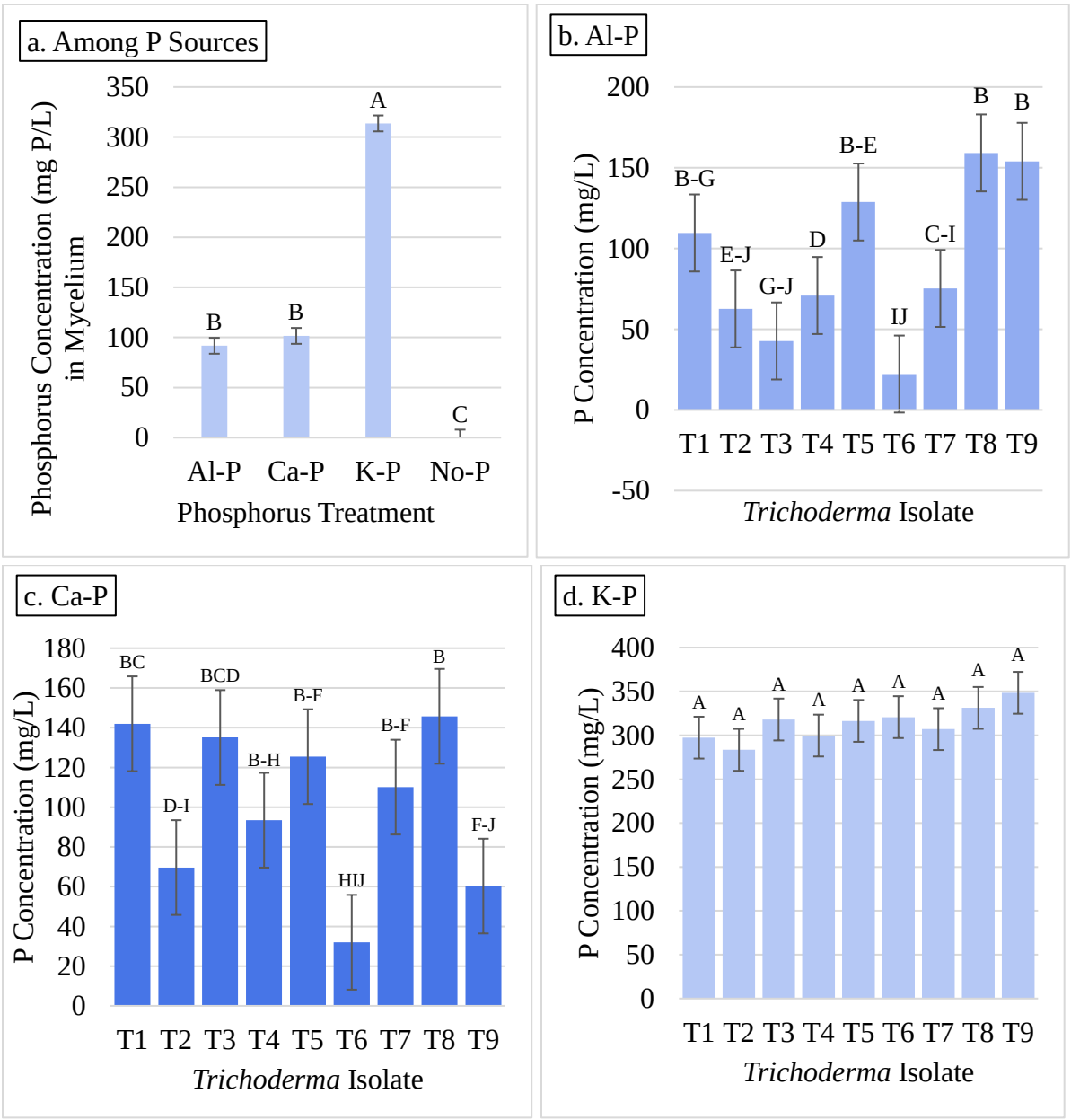

Figure 2.6: Mean phosphorus (P) concentration (mg P/L) among phosphorus sources (a), and *Trichoderma* isolates for each phosphorus source; Al-P (b), Ca-P (c), K-P (d), no-P (e). Representative of Objective 2, bars denoted with different letters are statistically significant at $P < 0.1$ as determined by Fisher's protected LSD.

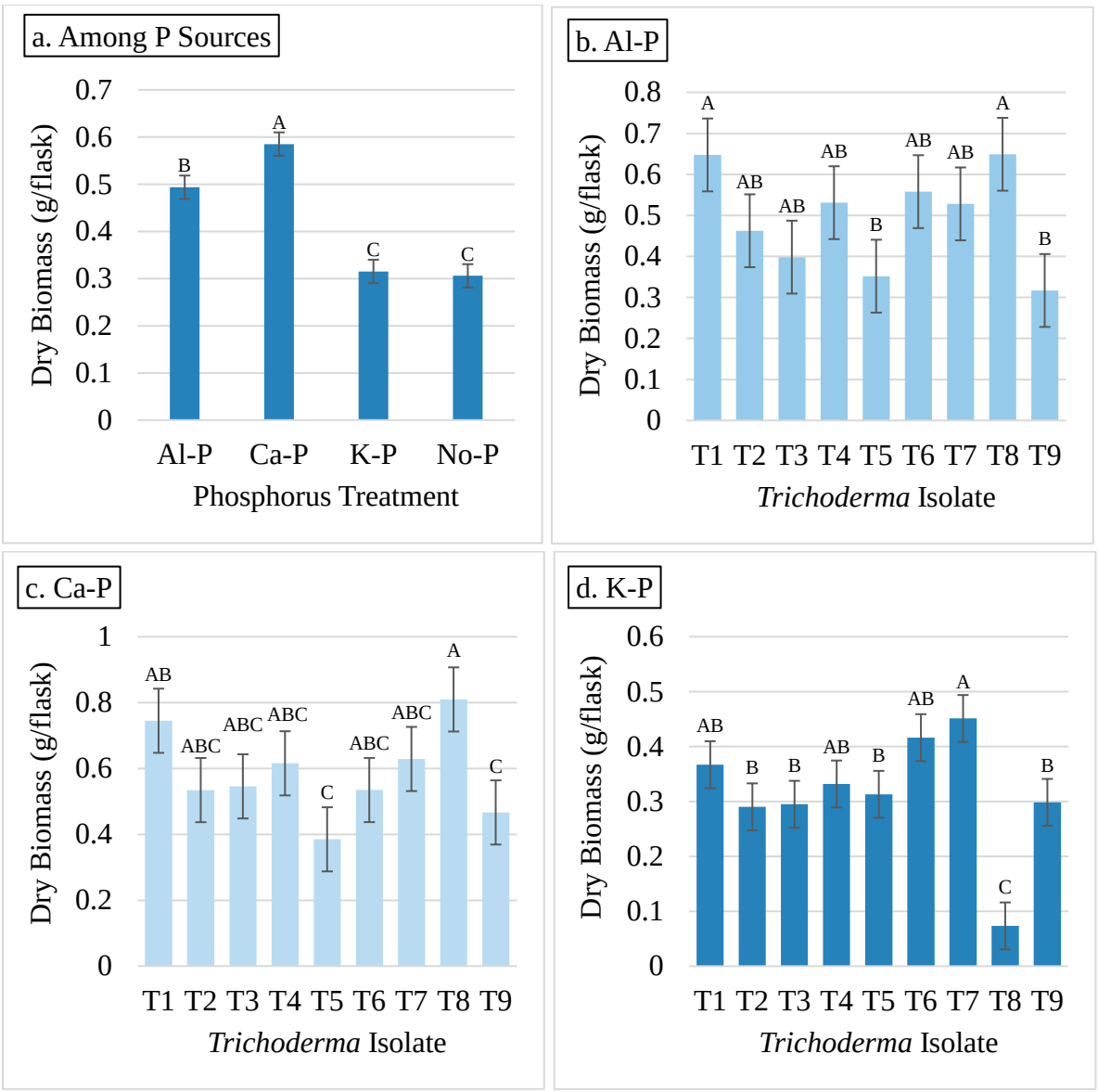

Figure 2.7: Mean mycelium biomass (g/flask) for Trichoderma isolates in PVK broth culture assay among phosphorus sources (a), and Trichoderma isolates for each phosphorus source; Al-P (b), Ca-P (c), K-P (d), no-P (e). Representative of Objective 2, bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

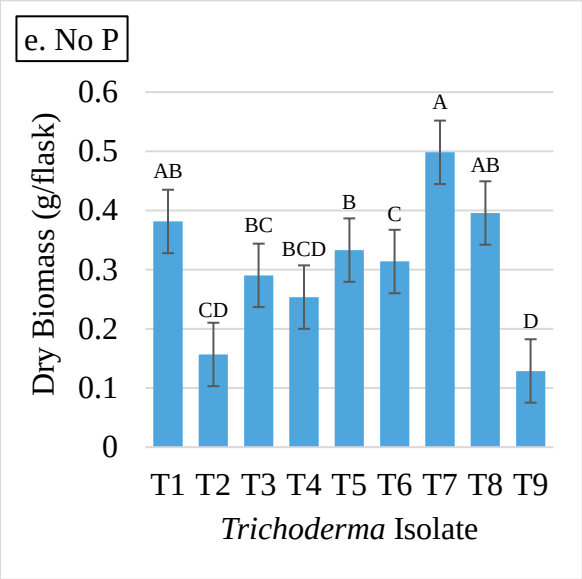

Figure 2.7: Continued.

biomass, isolates T5 (0.352 g/flask) and T9 (0.317 g/flask) had the lowest average biomass, with all other isolates having intermediate biomass. In PVK broth with Ca-P, mean dry biomass of *Trichoderma* was lowest for isolates T9 (0.467 g/flask) and T5 (0.385 g/flask), while the highest biomass was observed for isolate T8 (0.810 g/flask), with intermediate values for all other isolates (Fig. 2.7c). In PVK broth with K-P, isolate T8 (0.0734 g/flask) had the lowest biomass (Fig. 2.7d). The highest biomass was observed for isolate T7 (0.451 g/flask), while all other isolates grown in K-P broth were intermediate and statistically similar. In PVK broth with No-P, isolate T7 (0.494 g/flask) had the highest biomass, while the lowest biomass was observed for isolate T9 (0.129 g/flask) (Fig. 2.7e). In the dual culture assay, there were significant effects for *Trichoderma* isolate, phosphorus source, and the mean colony size ratio of *Trichoderma* to *Fol* on PVK agar (Fig 2.8). For Al-P, the ratio was highest for isolate T6 (2.4; indicating the *Trichoderma* isolate colony was, on average, 2.4x the size of the *Fol* colony) and T7 (2.3) (Fig 2.8a). The lowest ratio which was observed for isolates T3 (1.3) and T4 (1.58). On PVK agar with Ca-P, isolate T8 had the highest ratio of growth to *Fol* (1.7) and isolate T1 the lowest (0.8) (Fig. 2.8b). On PVK agar with K-P, isolate T6 (2.8) had the highest ratio, and the lowest ratio was observed for isolate T3 (0.95) (Fig. 2.8c). On PVK with no-P, the highest ratio was exhibited by isolate T1 (2.5) and the lowest by isolate T2 (1.1) (Fig. 2.8d).

Dual culture competition ratings on PVK agar with Al-P were similar for isolates T4, T5, T6, T7, and T8 which had the highest competitiveness ratings (Fig 2.9a). Isolates T3 and T2 had the lowest competition rating against *Fol* (Fig 2.9a). For PVK with Ca-P, isolates T2, T3, T4, and T5 were all statistically similar with high competitiveness ratings, although only statistically higher than isolate T7 which had the lowest competitiveness rating against *Fol* on PVK with Ca-P (Fig. 2.9b). For PVK with K-P, isolates T4, T6, T8, and T9 were all statistically similar with high competitiveness ratings, while T2 had the lowest competitiveness rating against *Fol* (Fig. 2.9c). Lastly, dual culture competitiveness ratings for PVK with no-P were similar for isolates T1, T4, and T8, which were higher than all other isolates which were similar, and no treatment groups had a competition rating of less than 50% (Fig. 2.9d).

Using similar methods of competition ratings, *Trichoderma* and *Fol* were also assigned growth ratings for sporulation and density (1 to 4) to determine growth percentages. There were significant differences in *Fol* between P sources on PVK for sporulation ($P < 0.001$) and density ($P < 0.0001$) and with combined factorials of phosphorus and isolates for sporulation ($P < 0.0001$) and density ($P < 0.0001$). However, there were no significant differences between isolates alone for sporulation or density. Growth between P sources was consistent within significant differences for both sporulation and density (Fig. 2.10a and b). *Fol* grown on Al-P had the highest sporulation (4) and density (4) which was similar to the second highest ratings in no-P treatments for sporulation (3) and density (4). These are in comparison to the third lowest ratings of K-P for sporulation (3) and density (3) while the lowest ratings were observed in Ca-P treatments for sporulation (2) and density (3).

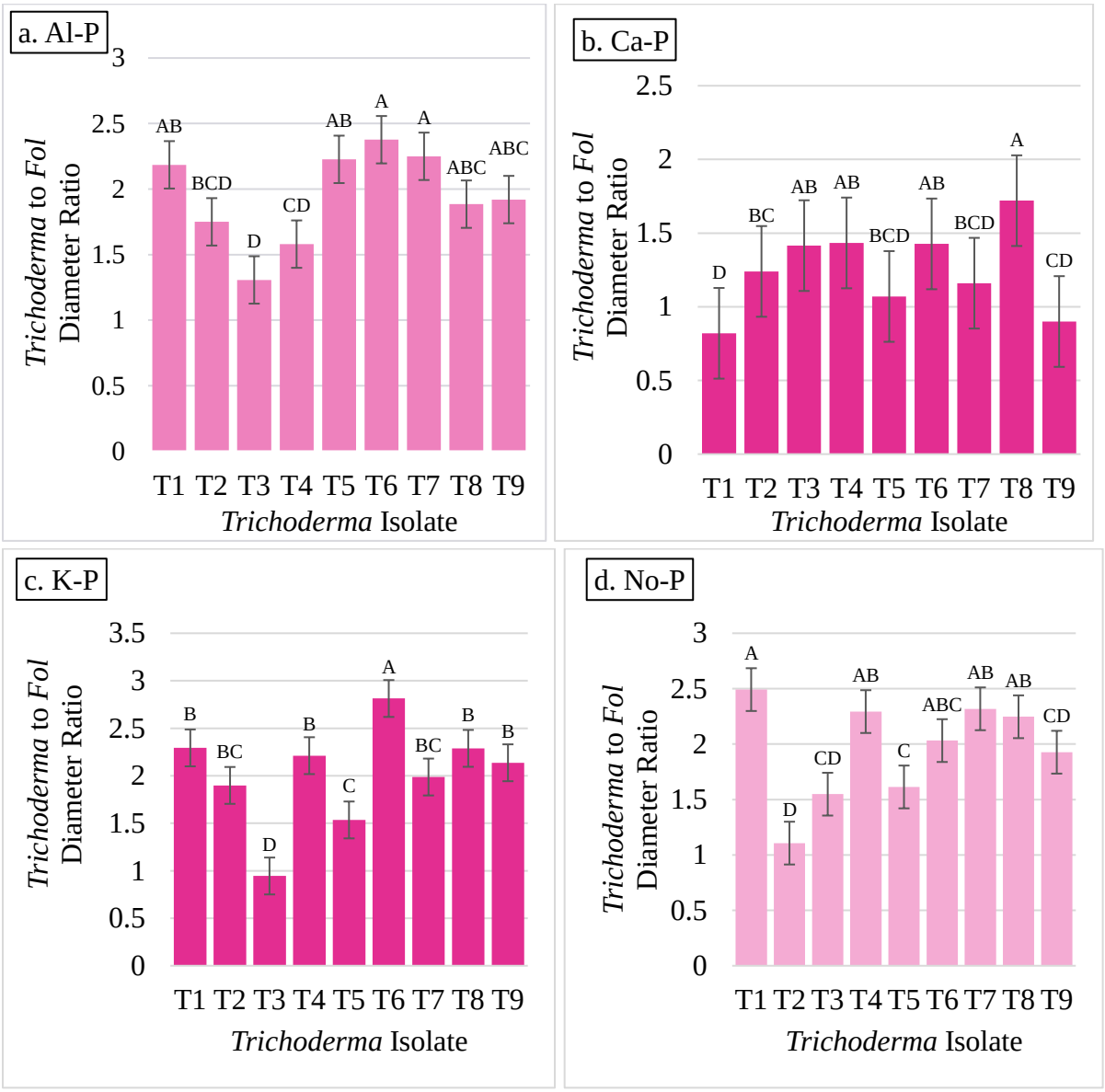

Figure 2.8: Representation of average ratio for *Trichoderma* diameter to *Fol* diameter grown on PVK agar medium with varying phosphorus sources; Al-P (A), Ca-P (B), K-P (C), and No-P (D). Representative of Objective 2, bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

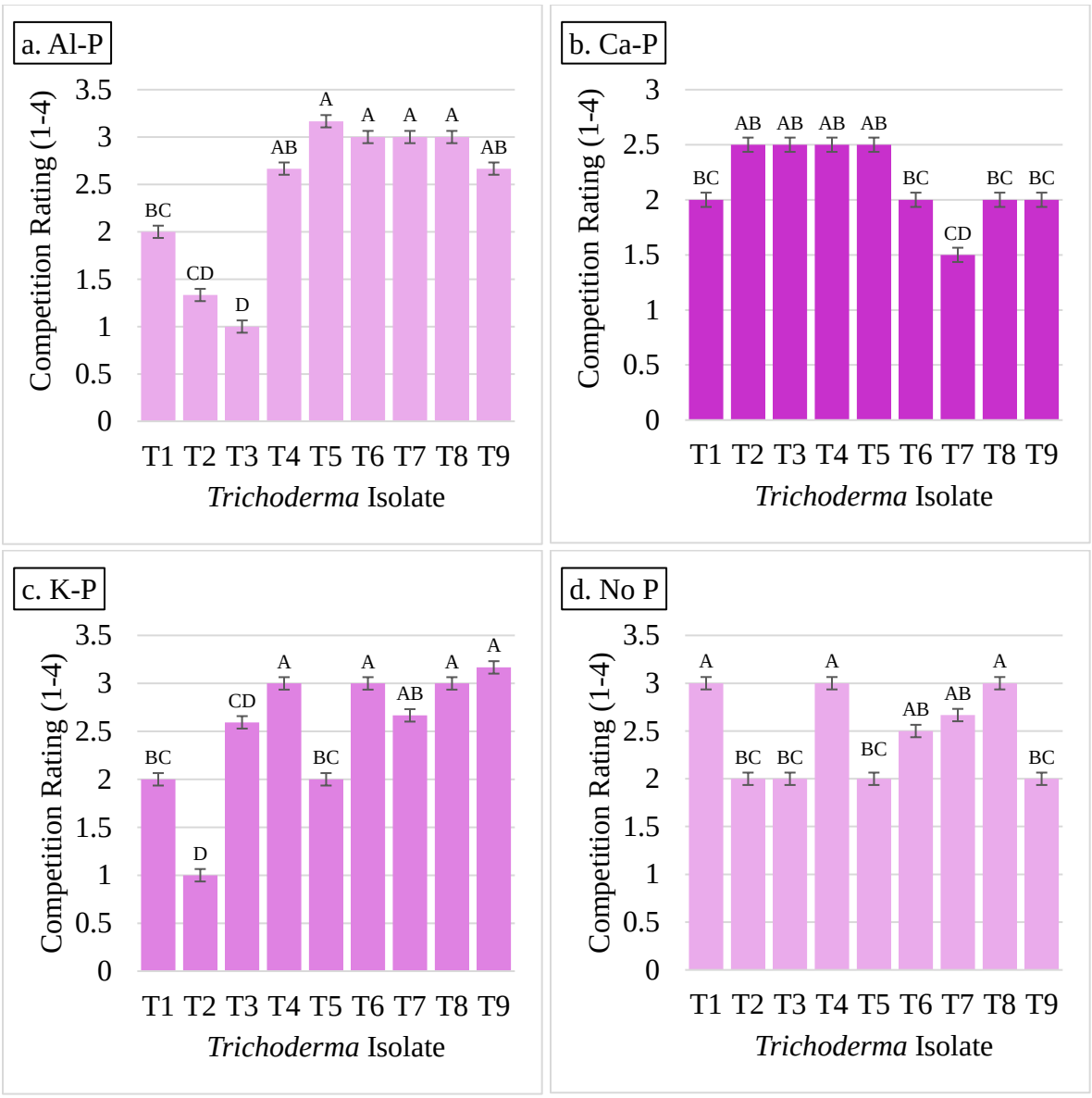

Figure 2.9: Representation of average competition (1 to 4 rating scale) of growth between Trichoderma treatments with *Fol* on PVK medium with varying P sources; Al-P(a), Ca-P (b), K-P (c), No-P (d). Representative of Objective 2, competition ratings are consistent with the rating scale in Figure 2.1. Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

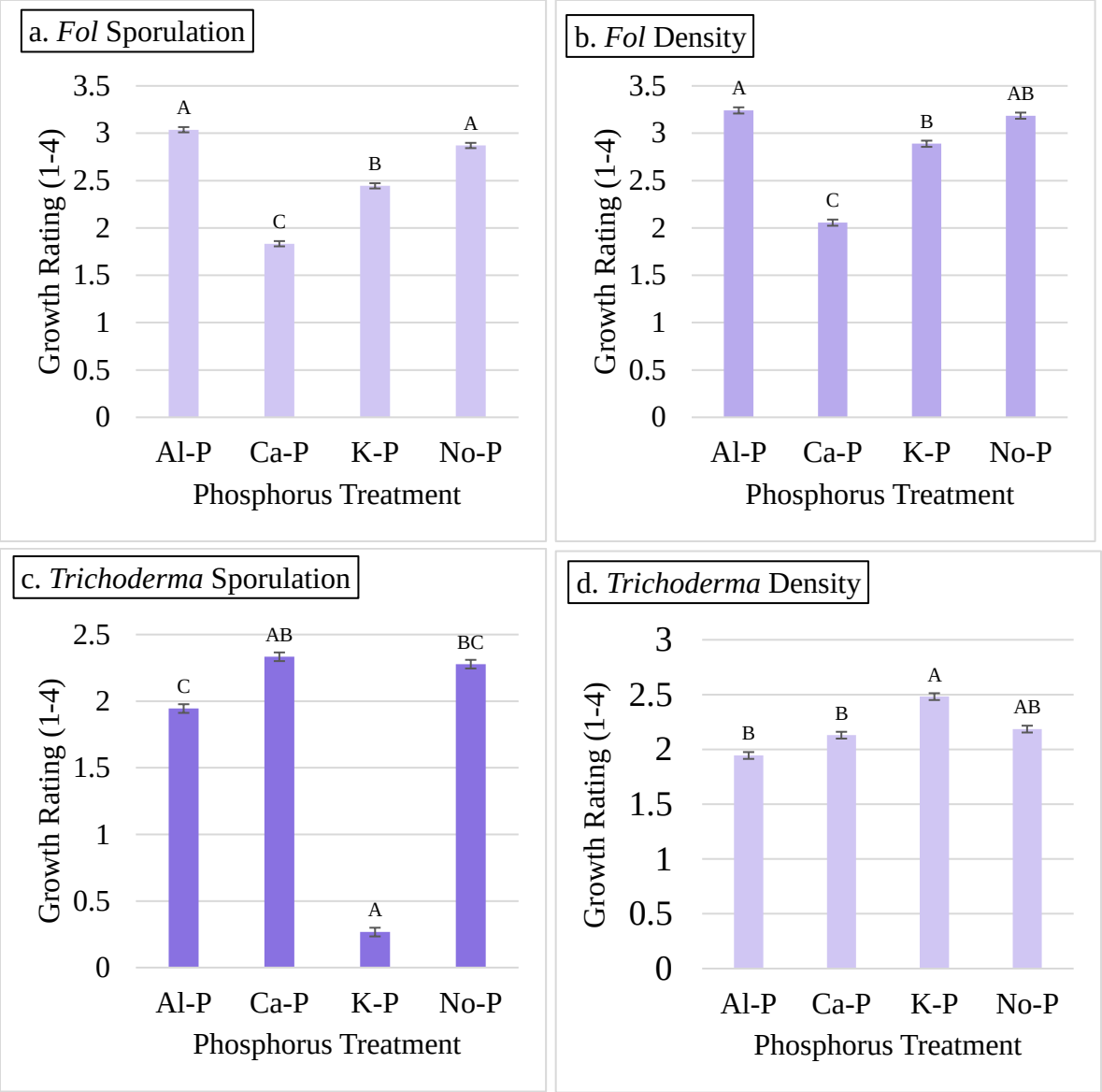

Figure 2.10: Representation of average growth ratings of sporulation and density (1-4 scale) for *Fol* and *Trichoderma* on PVK medium with varying P sources in dual culture assays; *Fol* sporulation (a) *Fol* density (b) *Trichoderma* sporulation (a) and *Trichoderma* density (b). Representative of Objective 2, growth ratings of density and sporulation are consistent with the rating scale in Figure 2.2. Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

For *Trichoderma* (Fig. 2.10c and d), there were significant differences in ratings of factorials for dual culture assays between isolates for sporulation ($P < 0.0001$) and density ($P < 0.0001$), between P sources for sporulation ($P = 0.002$) and density ($P = 0.02$) and of the interaction of P source and *Trichoderma* isolate for sporulation ($P = 0.004$) and density ($P < 0.0001$). Between P treatments, *Trichoderma* growth was highest for sporulation (3) and density (3) on K-P medias. The lowest ratings were observed on Al-P medias for sporulation (2) and density (48%) however, this density rating was statistically similar to that of Ca-P (3) and No-P (3). Sporulation on Ca-P (3) and No-P (3) were statistically similar.

In Table 2.2, color codes of *Trichoderma* strains and phosphorus sources on PVK from dual culture assays are represented. *Fol* color codes from the same study. These can be compared to the color codes of *Trichoderma* and *Fol* on PDA, represented in Table 2.3, which act as control groups and standards for sporulation/coloration of species. We observed coloration patterns between *Trichoderma* and P-source on PVK agar compared to PDA. *Trichoderma* on PDA had the darkest and most vibrant green colors, including dull green, grey-green, and olive green as generalized by the Methuen Handbook of Colour (Wanchester et al., 1967). *Trichoderma* isolates grown on PVK with Ca-P were the most similar to PDA compared to other PVK treatment groups (Al-P, K-P, No-P). Color patterns were often similar within Al-P, K-P, and No-P among isolates. However, these similarities were not consistent with *Trichoderma* diameter and *Fol* competition ratings. For example, *Fol* only had white coloring on PDA, while dual culture environments with various P sources and *Trichoderma* resulted in pink, red, and brown shades within *Fol* growth.

Discussion and Conclusions

As tomatoes are one of the most economically important crops, there is a substantial need for improved management strategies for biotic and abiotic pressures, such as *Fol*, and nutrients, such as phosphorus. While both factors can be very limiting to tomato production, beneficial fungi, such as *Trichoderma*, have the potential to act as biocontrol and biofertilizer enhancers for different crops and production settings. In Obj. 1, there were significant differences between *Trichoderma* isolates (T1 to T8) and control groups (C1 and C2) in tomato disease and drought tolerance, and total above ground biomass. We recorded the greatest aboveground biomass of tomato in isolate T3 compared to other *Trichoderma* treatments and control 2 (no *Trichoderma*/with *Fol*). Isolate T3 also had the lowest percentage of tissue yellowing between *Trichoderma* treatments, and a lower wilt rating. While isolate T5 had the second highest biomass, being statistically similar to isolate T3. However, drought stress and disease ratings of T5 were statistically similar to most other isolates. This suggests that mechanisms of disease control and growth promotion likely differ among isolates.

Table 2.2: Dominant colors of Trichoderma growth/sporulation grown on PVK in dual culture assays with Fol and varying P sources. These are compared to control groups of Trichoderma grown on PDA. Sample set number indicates dual culture correspondent with Fol in Table 3.2. All sample sets have 3 replicates. Color code/names from the Methuen Handbook of Colour (Wanscher et al., 1967).

Sample Set	Medium	P Treatment	Fungi	Color Code/Name
1	PVK	Ca	T1	• 1C4: pastel yellow • 28E7: grayish green • White
2	PVK	Al	T1	• 2A5: light yellow • White
3	PVK	K	T1	• 2A2: yellowish white/pale • White
4	PVK	No P	T1	• 27A2: greenish white • 27D7: grayish green • White
n/a	PDA	n/a	T1	• 2B2: yellowish white/pale • 27B4: grayish green • 27E4: dull green
5	PVK	Ca	T2	• 4A7: yellowish orange • 2A7: yellow • White
6	PVK	Al	T2	• 2A7: yellow • White

Table 2.2: Continued

7	PVK	K	T2	• 2A3: pale yellow • 27A3: pale green • White
8	PVK	No P	T2	• 2A7: yellow • White
n/a	PDA	n/a	T2	• 2B2: yellowish white/pale • 27B4: greenish gray • 27E4: dull green
9	PVK	Ca	T3	• 27D5: grayish green • White
10	PVK	Al	T3	• 27D6: grayish green • White
11	PVK	K	T3	• 27D4: dull green • White
12	PVK	No P	T3	• 27E7: grayish green • 27D4: dull green • White
n/a	PDA	n/a	T3	• 3E8,1E5: olive • 1B2: greenish gray • 27E4: dull green
13	PVK	Ca	T4	• White
14	PVK	Al	T4	• White
15	PVK	K	T4	• White

Table 2.2: Continued

16	PVK	No P	T4	• White
n/a	PDA	n/a	T4	• 26D4: dull green • 26D5, 26E4: grayish green • White
17	PVK	Ca	T5	• 2A7: yellow • 27E7: grayish green • White
18	PVK	Al	T5	• 2B4: grayish yellow • 27D6: grayish green • White
19	PVK	K	T5	• 2A6: yellow • 2B6,2C5: grayish yellow • White
20	PVK	No P	T5	• 27E7: grayish green • 27A2: greenish white • White
n/a	PDA	n/a	T5	• 26D4: dull green • 26B4: grayish green
21	PVK	Ca	T6	• 27E8: deep green • 27F8: dark green • White

Table 2.2: Continued

22	PVK	Al	T6	• 27D8: deep green • 27C5: grayish green • 2A3: pale yellow • White
23	PVK	K	T6	• 27E8: deep green • 27A2: greenish white • 2B5: grayish yellow • 2A3: yellowish white/pale • White
24	PVK	No P	T6	• 27B4: grayish green • 27E8: deep green • White
	PDA	n/a	T6	• 26D4: dull green • 26B4, 26E6: grayish green • 2B2: yellowish white/pale
25	PVK	Ca	T7	• 27F8: dark green • 27D5: grayish green • White
26	PVK	Al	T7	• 27C5: grayish green • 27E8: deep green • 30B5: grayish green • White

Table 2.2: Continued

27	PVK	K	T7	• 27E8: deep green • 27D5: grayish green • 2A5: light yellow • 2B5: grayish green • White
28	PVK	No P	T7	• 26E8: deep green • 26F8: dark green • 26B4: grayish green • 26A2: greenish white • White
n/a	PDA	n/a	T7	• 26D4: dull green • 26B4, 26E6: grayish green • 2B2: yellowish white/pale
29	PVK	Ca	T8	• 3A7: yellow • 27E7: grayish green • White
30	PVK	Al	T8	• 2A6: yellow • White
31	PVK	K	T8	• 3A5: light yellow • 27D5, 27E7: grayish green • White

Table 2.2: Continued

32	PVK	No P	T8	• 27A4: pastel green • 27A6: green • 27E7: grayish green • White
n/a	PDA	n/a	T8	• White • 2B2: yellowish white/pale • 26D4: dull green 26B4, 26E6: grayish green
33	PVK	Ca	T9	• 2A2: yellowish white/pale • 2A5: light yellow • White
34	PVK	Al	T9	• 2A2: yellowish white/pale • White
35	PVK	K	T9	• 2A2: yellowish white/pale • White
36	PVK	No P	T9	• White
n/a	PDA	n/a	T9	• White • 1E5,3E8: olive 27E4: dull green

Table 2.3: Dominant colors of Fol growth/sporulation grown on PVK in dual culture assays with Trichoderma and varying P sources. These are compared control groups of Fol grown on PDA. Sample set number indicates dual culture correspondent with Trichoderma from Table 3.1. All sample sets have 3 replicates. Color code/names from the Methuen Handbook of Colour (Wanscher, 1967).

Sample Set	Medium	P Treatment	Fungi	Color Code/Name
1	PVK	Ca	*Fol*	• White
2	PVK	Al	*Fol*	• White • 10B5,10C5: grayish red
3	PVK	K	*Fol*	• White • 10A2: red/pinkish white • 10B3: dull red
4	PVK	No P	*Fol*	• White • 10A2: red/pinkish white • 10C6: brownish red
5	PVK	Ca	*Fol*	• White
6	PVK	Al	*Fol*	• White • 10A2: red/pinkish white • 10D7: brownish red
7	PVK	K	*Fol*	• White • 10A2: red/pinkish white
8	PVK	No P	*Fol*	• White • 10D7: brownish red • 10B5: grayish red
9	PVK	Ca	*Fol*	• White
10		Al	*Fol*	• White • 10E6: violet red • 10B3: dull red

Table 2.3: Continued

11	PVK	K	*Fol*	• White • 10A2: red/pinkish white
12	PVK	No P	*Fol*	• White • 10E7: violet brown • 10B3: dull red
13	PVK	Ca	*Fol*	• White
14	PVK	Al	*Fol*	• White • 10C5: grayish red • 10A2: red/pinkish white
15	PVK	K	*Fol*	• White • 10A2: red/pinkish white
16	PVK	No P	*Fol*	• White • 10B4: dull red • 10C6,10D7: brownish red
17	PVK	Ca	*Fol*	• White
18	PVK	Al	*Fol*	• White • White • 10C5: grayish red • 10A2: red/pinkish white • 10C5: grayish red • 10D7: brownish red
19	PVK	K	*Fol*	• White • 10A2: red/pinkish white
20	PVK	No P	*Fol*	• White • 10B4: dull red • 10E7: violet brown
21		Ca	*Fol*	• White

Table 2.3: Continued

22	PVK	Al	*Fol*	• White • 10A2: red/pinkish white • 10C6: brownish red • 10B5: grayish red
23	PVK	K	*Fol*	• White • 10A2: red/pinkish white
24	PVK	No P	*Fol*	• White • 10A4: pastel red • 10E8: violet brown
25	PVK	Ca	*Fol*	• White
26	PVK	Al	*Fol*	• White • 10D7: brownish red • 10B7: red
27	PVK	K	*Fol*	• White • 10A2: red/pinkish white • 10B4: dull red
28	PVK	No P	*Fol*	• White • 10B4: dull red • 10E8: violet brown
29	PVK	Ca	*Fol*	• White
30	PVK	Al	*Fol*	• White • 10A4: pastel red • 10D8: brownish red
31	PVK	K	*Fol*	• White • 10A4: pastel red • 10C6: 10C6: brownish red

Table 2.3: Continued

32	PVK	No P	*Fol*	• White • 10A5: pastel red • 10D7: brownish red
33	PVK	Ca	*Fol*	• White
34	PVK	Al	*Fol*	• White • 10A4: pastel red • 10D7: brownish red
35	PVK	K	*Fol*	• 10A4: pastel red • 10C6,10D7: brownish red
36	PVK	No P	*Fol*	• White • 10A2: red/pinkish white • 10D7: brownish red
n/a	PDA	n/a	*Fol*	• White

Studies show that using *Trichoderma* treatments against *Fol* in tomato can reduce disease and increase plant growth (Sallam et al., 2019), our results support this idea, and show there is a significant difference between tomato biomass, with variation between *Trichoderma* treatments and overall greater results than control 2 (no *Trichoderma*/with *Fol*). We hypothesized that the ability of *Trichoderma* isolates to reduce symptoms of *Fol* on 'Valencia' tomato compared to a *Fol*-inoculated control will vary among isolates, with some native isolates being equivalent to a non-*Fol* inoculated control. While isolates did vary in ability to reduce disease symptoms, which is supported in disease ratings and overall plant biomass, there were no instances where *Trichoderma* treatment groups were statistically similar to C1 (no *Trichoderma*/no *Fol*) for all response variables.

Our broth and dual culture assays supported the hypothesis that *Trichoderma* isolates would have varying outcomes in regard to P solubilization potential, pathogenic competition against *Fol*, and differing morphologies as a result of different growth environments. In our broth assay, we expected that *Trichoderma* grown in K-P would have the highest biomass, as this is the most readily available source. However, Ca-P treatments had the greatest biomass while Al-P had the second largest biomass. This is in comparison to our no-P control which, as expected, had the lowest average biomass, although it was statistically similar to K-P. As the average biomass of Al-P was significantly greater than K-P, this supports the idea that *Trichoderma* isolates have the potential to increase P availability and plant growth through combined mechanisms in highly weathered, acidic soils where Al-P is predominant. Looking at the results of specific isolates, the dry biomass of isolates T1 and T8 were both statistically similar and the greatest values compared to the growth of other isolates grown in Al-P broth.

Looking at the average P concentrations by phosphorus source, K-P treatments had the highest P concentration. Comparatively, P concentrations of Al-P and Ca-P were both significantly similar to one another and significantly lower than K-P. This supports that *Trichoderma* can aid in solubilization of P in soil environments where plant available P is limited (Rinu, 2013; Bader et al., 2019). Isolate T6 was consistent in P solubilization in both Al-P and Ca-P broth treatment groups, while T3 had greater solubilization potential in Al-P broth and T2 exhibited great solubilization in Ca-P broth. In contrast, T8 consistently had one of the highest P concentrations in Al-P and Ca-P broths. This result is somewhat contradictory to our biomass results, as T8 also showed substantial growth compared to other isolates, suggesting growth could be independent of phosphorus availability for some isolates.

In dual culture assays with varying P sources on agar media with Al-P, the largest growth ratios were observed with isolates T6 and T7, which were statistically similar. In contrast, the lowest growth ratio resulted from T3 when grown in PVK broth with Al-P. The PVK broth with K-P had the greatest growth ratios compared to all other PVK treatments, with some isolates having greater growth ratios in No-P treated PVK. We also observed the highest density and sporulation of isolates grown on K-P, while lowest averages for sporulation and density were observed on Al-P treatments. This aligns with our original idea that *Trichoderma* would have the

best growth on K-P, which is the most available. However, this result is in contrast with our broth assay, which had the greatest mycelium biomass in PVK treated with Al-P. Contradictions in results of *Trichoderma* growth on PVK agar media compared to broth assays may be due to competing interactions of *Fol* or differences in the relative importance of different P-solubilization mechanisms in broth vs. agar media.

Differences in morphology of cultures grown within the dual assay can indicate signs of competition between antagonisms of *Trichoderma* and *Fol*, along with effects of phosphorus sources through benefits or limitations. Differences in color codes support the idea that *Trichoderma* is being affected by the culture plate environment on PVK with *Fol*, as there is variation in color between treatment groups, also in comparison to PDA controls.

Results of the objective 1 pot study and objective 2 lab assays show potential for *Trichoderma* to increase tomato growth and resistance. Our results showed significant differences between *Trichoderma* growth patterns when exposed to varying P sources and *Fol* in different lab culture media environments. While treatment results were often consistent within studies, the amount of variation in *Trichoderma* responses suggests more research is needed to better understand the relationships between tomato, phosphorus, *Trichoderma* isolates, and *Fol*. Results also show that *Trichoderma* has potential to solubilize different forms of P, including Al-P which is not readily available for plant use and uptake. While this could be beneficial to growers in areas with limited P availability, it may also influence biocontrol activity of *Trichoderma* as P availability varies in the growing environment which, as a result, could cause *Trichoderma* to be more or less parasitic/competitive with *Fol*. We also observed *Fol* exhibiting different growth characteristics when exposed to different P sources in dual culture assays. Experiments should be conducted to better understand the relationships of *Trichoderma* isolates, *Fol*, and P in greenhouse or field studies to determine how *Trichoderma* can increase P uptake and plant growth, and how P sources can also influence *Fol* disease potential.

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CHAPTER THREE

COMPARING NATIVE *TRICHODERMA* SPP. FOR PHOSPHORUS SOLUBILIZATION AND BIOCONTROL POTENTIAL OF *FUSARIUM OXYSPORUM F. SP. LYCOPERSICI* WITH GENETICALLY DIVERSE TOMATOES GROWN IN TENNESSEE ULTISOLS

Abstract

Beneficial soil fungi, such as the genus *Trichoderma*, are known to improve crop growth and stress tolerance through multiple mechanisms. For example, many *Trichoderma* isolates can solubilize poorly soluble soil nutrients such as phosphorus (P). In addition, many *Trichoderma* isolates are recognized for their ability to induce plant resistance to soilborne pathogens and parasitize pathogenic microbes, such as *Fusarium oxysporum* f. sp. *lycopersici (Fol)*, which causes fusarium wilt. At the same time, there is little information on how *Trichoderma*-induced benefits to crops interact, or the magnitude of the benefits from nutrient solubilization, especially in the case of genetically diverse crops, such as tomato. In objective 1, we evaluated native *Trichoderma* isolates (T3; *T. arundinaceum*, and T5; a *T. harzianum* species complex isolate) for potential to solubilize poorly soluble forms of soil P and increase crop P uptake in genetically diverse tomatoes with varying levels of domestication (*Solanum habrochaites, S. pimpinellifolium, S. lycopersicum* var. *cerasiforme, S. lycopersicum* cv. Valencia, and *S. lycopersicum* cv. Clementine). This study was conducted under greenhouse pot conditions. Tomatoes were treated with *Trichoderma* (T3 or T5) or no *Trichoderma* (control) and one of four P treatments (high and low concentrations of aluminum phosphate; potassium phosphate, or no phosphorus). Results indicate that tomato P uptake varied primarily by genotype and P source. *Trichoderma* isolate had limited effects on tomato P uptake, but isolate effects on soil inorganic P varied by tomato genotype and P source. Similarly, in objective 2, we aimed to analyze the relationships of tomato type and P fertilization with the inoculation of *Trichoderma* for P solubilization potential and biocontrol potential against Fusarium wilt *(Fol).* Under greenhouse pot conditions, tomato types *S. l. cerasiforme* (cv. Matt's Wild Cherry*)* and heirloom (cv. Valencia) were evaluated with *Trichoderma* (T5) and fertilization with aluminum phosphate or no phosphate, in combination with the *Fol* inoculation or a non-inoculated control. Results indicate that T5 increased solubilization of Al-P as observed in soil inorganic P measurements. Evidence also indicated greater resilience and lower disease ratings in tomato with Al-P fertilization compared to non-fertilized treatment groups.

Introduction

Tomatoes, the second most widely cultivated vegetable, have undergone extensive selective breeding, leading to various types from wild relatives to modern hybrids (McGovern et al., 2015; Guan et al., 2018; Cao et al., 2018). Domestication and breeding have caused "domestication syndrome," resulting in a 95% loss of natural traits (Bergougnoux et al., 2013). This genetic reduction often affects plant-microbe interactions, nutrient use efficiency, and resilience to environmental stressors (Banani et al., 2013; Cordova-Campos et al., 2012; Smulders et al., 2020).

Fusarium species, such *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), are responsible for fusarium wilt disease, which causes significant damage to various crops globally (Duyvesteijn et al., 2005). In tomatoes, *Fol* races 1, 2, and 3 lead to damping off, basal stem necrosis, yellowing foliage, and wilting, eventually causing plant death. Asexual reproduction and survival structures

of *Fol* enhance its resilience and ability to endure harsh conditions (McGovern, 2015; Michielse and Rep, 2009). As a wilt pathogen, *Fol* clogs xylem cells, disrupting water flow and causing symptoms like one-sided wilt and yellowing, especially in warm temperatures (>28°C) (Duyvesteijn et al., 2005; McGovern et al., 2015). The pathogen's resilience and limited control options heighten the need for effective management strategies (Shrestha et al., 2021; Yergeau et al., 2006). *Trichoderma* is a genus of opportunistic, free-living fungi found in soils worldwide, known for its rapid growth and adaptability to various environmental conditions (Harman, 2004; Cai and Chen, 2014; Hoyos-Carvajal et al., 2009). Due to its benefits in plant production, including enhancing growth and stress resilience, and its ability to parasitize plant pathogens, *Trichoderma* is valuable for biocontrol applications (Nawrocka et al., 2013; Bader et al., 2019; Asghar and Kataoka, 2021; Tucci et al., 2011). Various *Trichoderma* strains can colonize plant roots and shoots, improving plant health and productivity (Harman et al., 2004).

Studies reveal a direct link between plant domestication, resilience, and soil-fungi interactions. Banani et al., (2014) found that the effectiveness of *Trichoderma* against downy mildew varied among grape (*Vitis* spp.) cultivars. For tomatoes, wild relatives showed stronger associations with beneficial soil bacteria compared to landraces and modern varieties (Smulders et al., 2020). Jaiswal et al. (2020) reported that wild tomato relatives had better resistance to *Botrytis cinerea* and were more responsive to *Trichoderma* benefits, such as increased shoot biomass and pathogen suppression, compared to landraces and modern cultivars. The effectiveness of *Trichoderma* also declined along the domestication gradient. Abiotic factors, like soil nutrients, also influence these plant-microbe interactions (Cordova-Campos et al., 2012).

Tomato crops, which produce high biomass, have high nutrient demands, particularly for phosphorus (P), a critical but finite nutrient (Balemi and Negisho, 2012). Over 40% of global soils are P deficient, especially in regions with highly-weathered, acidic soils like Ultisols and Oxisols in the southeastern USA, where P is strongly bound to clay minerals or iron and aluminum oxides (Rinu et al., 2013; Bader et al., 2019). The ability of plants to uptake phosphorus varies by species and genotype, with some tomato varieties showing a 77% increase in dry weight under low P conditions (Dixon et al., 2020). Effective P use in tomatoes involves strategies such as rapid P sensing, optimized root growth, and microbial interactions. Understanding how plant breeding and domestication affect P use through plant morphology, metabolism, and beneficial microbial relationships is crucial for improving P efficiency (Balemi and Negisho, 2012; Hoyos-Carvajal et al., 2009; Dixon et al., 2020). Microorganisms, particularly fungi, play a crucial role in the biogeochemical cycling of P in agroecosystems. Phosphate-solubilizing fungi (PSF) are especially effective for nutrient management due to their larger habitat range and greater resilience compared to bacteria (Rinu et al., 2013; Doilom et al., 2020; Bononi et al., 2020). The PSF enhance P availability by producing organic acids that convert soil P into readily accessible forms for plant uptake (Bononi et al., 2020). However, the

effectiveness of PSF in solubilizing P depends on site-specific soil conditions, such as the forms of phosphorus compounds and soil pH (Ngo et al., 2021; Elias et al., 2016).

Trichoderma, a PSF, can be beneficial in agricultural environments, particularly in P-deficient soils. A study on *Trichoderma* with soybean and various P fertilizers in the Amazon's acidic, weathered soils found that two *Trichoderma* isolates (AMS 34.39 and AMS 31.15) enhanced P solubilizing enzyme activity by 16 to17% with rock phosphate and 10 to15% with triple superphosphate. Rock phosphate showed the highest enzymatic activity due to its lower availability, which stimulated greater microbial activity. Optimal soybean biomass increases were observed at 70 kg P/ha, with notable improvements in plant height at P rates of 50, 70, and 90 kg P/ha when combined with *Trichoderma*. Overall, *Trichoderma* significantly boosted plant growth and P availability compared to treatments without the fungi (Bononi et al., 2020). *Trichoderma* has potential to enhance nutrient availability, particularly phosphorus (P), through mineralization and solubilization in the rhizosphere (Cai and Chen, 2014). It can also parasitize pathogenic fungi with antibiotic production and improve plant growth by enhancing root structure (Harman, 2004; Bader et al., 2019). Fertilization impacts plant disease resistance, with studies indicating that while P fertilization slightly increases *Fusarium* disease severity, it does not match the impact of different forms of nitrogen (N) fertilization. The highest disease severity in some tomato plants occurs with combined N and P fertilization (Veresoglou et al., 2012). However, there is limited information on the effectiveness of *Trichoderma* in P uptake and disease management in highly weathered, acidic soils, necessitating further research on how *Trichoderma* affects nutrient availability and disease severity in tomato crops (Srihuttagum et al., 1989).

To better understand how the ability of native *Trichoderma* isolates to solubilize soil P affects biocontrol and plant nutrient uptake in genetically diverse tomato, two greenhouse trials were conducted to meet two objectives. In objective 1, we evaluated two *Trichoderma* isolates (T3; *T. arundinaceum* and T5; *T. harzianum*) with differing P-solubilization potential on tomato P-uptake in a greenhouse trial of five diverse tomato genotypes/wild relatives and varying P fertilization. We hypothesized that high P-solubilizing *Trichoderma* would increase tomato biomass and P uptake when the tomato was grown in low-P availability substrates, but the magnitude of effects would differ among tomato germplasm and be more pronounced in tomato wild relatives. In objective 2, we evaluated isolate T5 (*T. harzianum*) for P-solubilization potential and biocontrol interactions (+/- *Fol*) with P-uptake and availability in greenhouse trials with two different tomato genotypes (cv. Matt's Wild Cherry (*S. lycopersicum* var. *cerasiforme;* second domestication) and cv. Valencia (*S. lycopersicum*; heirloom). We hypothesized that tomato biomass, performance, and wilt resistance would improve under low soil P availability when inoculated with high P-solubilizing *Trichoderma*.

Methodology

Objective 1. Eight native *Trichoderma* isolates and one commercial *Trichoderma* isolate were analyzed in a previous study (Dalton, Ch. 1) for P solubilization potential to determine two

isolates that differed in potential to solubilize P in greenhouse and lab studies for this study. This data indicated that *Trichoderma* isolates T3 (*T. arundinaceum*) and T5 (*T. harzianum*) had potential for improving P availability from aluminum phosphate in acidic clay soils, as well as relatively high biocontrol potential.

Trichoderma isolates were cultured on potato dextrose agar (PDA); 31.2 g / 800 mL sterile deionized water with 0.4 mg rifampicin in 85-mm Petri dishes. *Trichoderma* was grown for 5 to 7 days under continuous, fluorescent lighting at room temperature (~22°C) to allow for dense, hyphal growth and sporulation to occur (Jaiswal, 2020). After 5 to 7 days, *Trichoderma* plates with dense, green conidial growth were scraped with a sterile toothpick (areas of 5 to 7 mm diam.) were deposited into a suspension deposited into a suspension using a stock solution of 0.1% Tween-twenty (Thermo-Fisher, Waltham, MA) in DI water to create a suspension of 25% stock to 75% DI water. The concentration of conidia within each solution was determined under a light microscope with a hemacytometer. Suspensions were then diluted in an aqueous solution of 1% carboxymethylcellulose to achieve a final concentration of 1×10^6 spores/mL (Shrestha, 2020).

Tomato seeds of five genotypes representative of a domestication continuum (*Solanum habrochaites, S. pimpinellifolium, S. lycopersicum* var. *cerasiforme, S. lycopersicum* cv. Valencia, and *S. lycopersicum* cv. Clementine) (Table 3.1) were surface sterilized by soaking in 3% hydrogen peroxide for 2 min, rinsed with sterile water, and air-dried for 10 min under a biosafety cabinet. Seeds were soaked in *Trichoderma* treatment suspensions for 15 min, while control seeds were soaked in the same concentration solution with no spores. Treated seeds were planted in organic medium (Lambert Organic All-Purpose Mix, Lambert, Québec, Canada) and grown in a greenhouse for 4 weeks in seedling trays prior to being transplanted. Plant establishment rates were recorded as live plants in week 4 before being transplanted into experimental pots. A total of 180 pots were used per trial (Fig 3.1). Variables included tomato type (Table 3.1) , two *Trichoderma* isolates (*T. arundinaceum,* T3 and *T. harzianum,* T5*)* and a non-inoculated control, and three P amendments (mono-potassium phosphate (KH_2PO_4) at 0.57 per 1,300 g soil and aluminum-phosphate ($AlPO_4$) with low concentration of 0.256 g per 1.300 g of soil and high concentration of 0.5112 g per 1,300 g of soil, plus a control with no added P. Pots of 16-cm-diam. were filled with 1,300 g of 50% sand and 50% field soil (UT Organic Research Unit). To prevent nutrient deficiency, soil was also amended with standard fertilizers; potassium nitrate (KNO_3) at 0.719 g per 1,300 g of soil and Micromax Micronutrients Granular (calcium; 6%, magnesium; 3%, sulfur boron; 0.1%, copper; 1%, water soluble copper iron; 17%, water soluble iron manganese; 2.5%, water soluble manganese molybdenum; 0.05%, zinc; 1%, water soluble zinc derivative; 1%) at 1 g per 1,300 g of soil. Soil samples were collected before transplanting and after harvest to evaluate soil pH because soil pH affects chemical properties of P availability and soil phosphatase activity (Rihn et al., 2013). After 9 weeks of growing in the greenhouse (average temperature 24° C) plant height and stem caliper were measured. Tomato plants were removed from pots, cleaned, and roots separated from shoot tissue. Above ground

Table 3.1: Identification information of tomato species for Objective 1.

Scientific Name	Level of Domestication
Solanum habrochaites	Wild tomato
Solanum pimpinellifolium	First domestication
Solanum lycopersicum var. *cerasiforme* cv 'Matt's Wild Cherry'	Second domestication
Solanum lycopersicum cv 'Valencia'	Heirloom
Solanum lycopersicum cv 'Clementine'	Modern hybrid

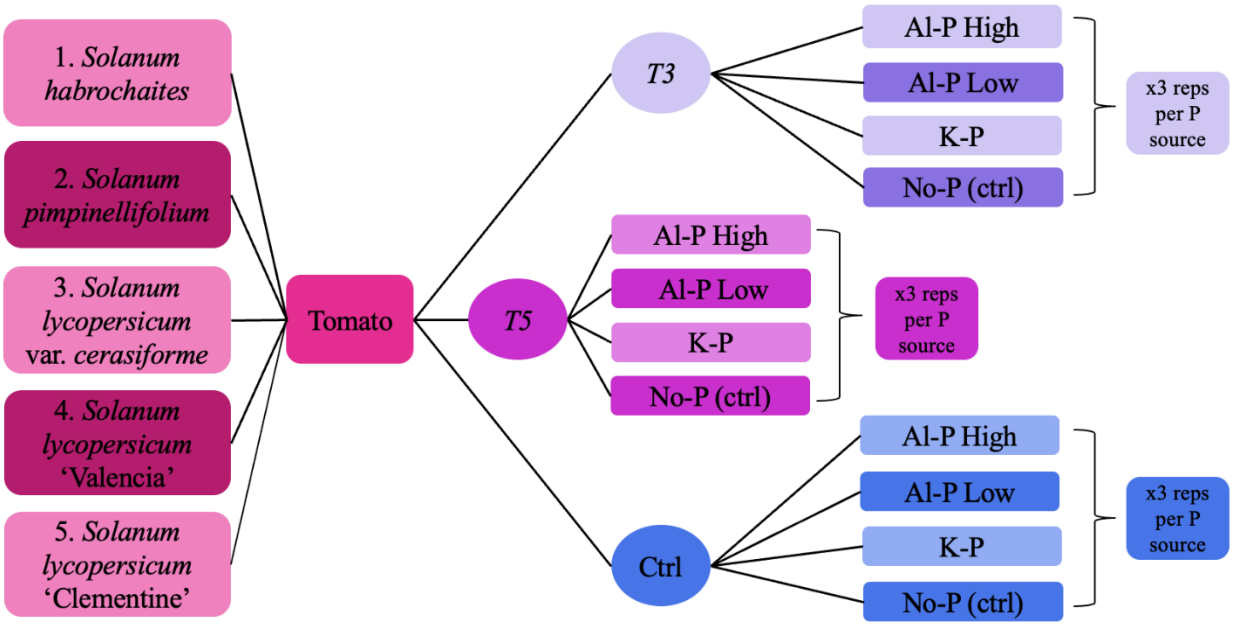

Figure 3.1: Experimental design for Objective 1: tomato types in order of domestication (1-5), *Trichoderma* treatments (T3; *T. aridunaceaum*, T5; *T. harzianum* species complex, and control) and phosphorus treatments (Al-P high, Al-P low, K-P, and No-P controls). Each treatment group was replicated 3 times, for a total of 180 pots per trial, with a total of two trials.

tissues were oven-dried at 65°C for 48 h prior to recording dry biomass. In brief, tissue samples were finely ground, and 2.5 g of each plant was collected and analyzed following microwave digestion by Inductively Coupled Plasma (ICP) spectroscopy (Water's Lab, Owensboro, KY.)

To assay tomato root systems for colonization by *Trichoderma* spp., from each plant sample fine tertiary roots were clipped at 1 to 2 cm lengths and arranged in 10 evenly spaced clusters on *Trichoderma* semi-selective medium (TSM); 31.2 g potato dextrose agar, 0.15 g rose bengal, 0.3 g streptomycin, 0.3 g chloramphenicol per 800 mL H_2O (Askew and Laing, 1993; Williams et al., 2003). Photo documentation was collected after 3, 5, and 7 days of culture growth for quantitative assessment. Approximately 100 g of soil was collected after 9 weeks from each pot to evaluate soluble inorganic P (D'Angelo et al., 2001.) Of these soil samples, 5 g were added to a 50 mL centrifuge tube and acidified with 20.0 mL of 0.0125 M H_2SO_4 + 0.05 M HCl solution (Mehlich 1), shaken 5 min on a shaker at 180 rpm, and centrifuged for 5 min at 3,500 rpm. Samples were filtered (Whatman No.2 filter paper) and stored at 18°C prior to analysis for P concentration. In brief, acidified samples were reacted with ammonium molybdate tetrahydrate and then malachite green carbinol hydrochloride in polyvinyl alcohol. The concentrations of inorganic P in broth solution at the end of incubation were determined by measuring absorbance at 630 nm, then comparing absorbance to a standard curve.

Select samples were evaluated for acid phosphatase activity in soil (Weaver et al., 1994). This included soils from cv. Valencia with all *Trichoderma* treatments (T3, T5) and control and fertilization treatments of Al-low and No-P. Soil (1-g) was added to a 50-mL centrifuge tube with 0.2 mL toluene, 4 mL modified universal buffer [12.1 g (hydroxymethyl) aminomethane; 11.6 g maleic acid; 14.0 g citric acid; 6.3 g boric acid in 488 mL of 1 N sodium hydroxide (NaOH) and diluted in 1 L of water], and 1 mL of p-nitrophenyl phosphate solution. This mixture was incubated for 1 h before adding 1 mL of 0.5 M CaCl2 and 4 mL of 0.5 M NaOH, then filtered through a Whatman no. 2 filter paper. Samples were measured using a spectrophotometer for yellow color intensity and read at 400 nm (Weaver et al., 1994).

A mixed model factorial analysis of variance (ANOVA; Statistical Analysis Software, version 9.3) for treatment (tomato type, P source, *Trichoderma* isolates and controls, and their interactions) effects on response variables was conducted. Data were evaluated for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) and data were transformed to address non-normal distribution and unequal variances of residual error where appropriate. "Trial" was treated as a random factor. Fisher's protected Least Significant Difference (P-LSD) test was used to separate means at 5% significance level; untransformed means and standard errors are reported. Data were pooled for analysis.

Objective 2. Tomato seeds of five genotypes representative of a domestication continuum (*Solanum habrochaites*, *S. pimpinellifolium*, *S. lycopersicum* var. *cerasiforme*, *S. lycopersicum* cv. Valencia, and *S. lycopersicum* cv. Clementine) (Table 3.1) were surface sterilized by soaking in 3% hydrogen peroxide for 2 min, rinsed with sterile water, and air-dried for 10 min under a biosafety cabinet. Seeds were soaked in *Trichoderma* treatment suspensions

for 15 min, while control seeds were soaked in the same concentration solution with no spores. Treated seeds were planted in organic medium (Lambert Organic All-Purpose Mix, Lambert, Québec, Canada) and grown in a greenhouse for 4 weeks in seedling trays prior to being transplanted. Plant establishment rates were recorded as live plants in week 4 before being transplanted into experimental pots. A total of 180 pots were used per trial (Table 3.2). Variables included tomato type (Table 3.1) , two *Trichoderma* isolates (*T. arundinaceum*, T3 and *T. harzianum*, T5*)* and a non-inoculated control, and three P amendments (mono-potassium phosphate (KH_2PO_4) at 0.57 per 1,300 g soil and aluminum-phosphate ($AlPO_4$) with low concentration of 0.256 g per 1.300 g of soil and high concentration of 0.511 g per 1,300 g of soil, plus a control with no added P. Pots of 16-cm-diam. were filled with 1,300 g of 50% sand and 50% field soil (UT Organic Research Unit). To prevent nutrient deficiency, soil was also amended with standard fertilizers; potassium nitrate (KNO_3) at 0.719 g per 1,300 g of soil and Micromax Micronutrients Granular (calcium; 6%, magnesium; 3%, sulfur boron; 0.1%, copper; 1%, water soluble copper iron; 17%, water soluble iron manganese; 2.5%, water soluble manganese molybdenum; 0.05%, zinc; 1%, water soluble zinc derivative; 1%) at 1 g per 1,300 g of soil. Soil samples were collected before transplanting and after harvest to evaluate soil pH because soil pH affects chemical properties of P availability and soil phosphatase activity (Rihn et al., 2013). After 9 weeks of growing in the greenhouse (average temperature 24° C) plant height and stem caliper were measured. Tomato plants were removed from pots, cleaned, and roots separated from shoot tissue. Above ground tissues were oven-dried at 65°C for 48 h prior to recording dry biomass. In brief, tissue samples were finely ground, and 2.5 g of each plant was collected and analyzed following microwave digestion by Inductively Coupled Plasma (ICO) spectroscopy (Walter's Lab, Owensboro, KY.)

To assay tomato root systems for colonization by *Trichoderma* spp., from each plant sample fine tertiary roots were clipped at 1 to 2 cm lengths and arranged in 10 evenly spaced clusters on *Trichoderma* selective medium (TSM); 31.2 g potato dextrose agar, 0.15 g rose bengal, 0.3 g streptomycin, 0.3 g chloramphenicol per 800 mL H_2O (Askew and Laing, 1993; Williams et al., 2003). Photo documentation was collected after 3, 5, and 7 days of culture growth for quantitative assessment. Approximately 100 g of soil was collected after 9 weeks from each pot to evaluate soluble inorganic P (D'Angelo et al., 2001.) Of these soil samples, 5 g were added to a 50 mL centrifuge tube and acidified with 20.0 mL of 0.0125 M H_2SO_4 + 0.05 M HCl solution (Mehlich 1), shaken 5 min on a shaker at 180 rpm, and centrifuged for 5 min at 3,500 rpm. Samples were filtered (Whatman No.2 filter paper) and stored at -37°C prior to analysis for P concentration. In brief, acidified samples were reacted with ammonium molybdate tetrahydrate and then malachite green carbinol hydrochloride in polyvinyl alcohol. The concentrations of inorganic P in broth solution at the end of incubation were determined by measuring absorbance at 630 nm, then comparing absorbance to a standard curve.

Select samples were evaluated for acid phosphatase activity in soil (Weaver et al., 1994). This included soils from cv. Valencia with all *Trichoderma* treatments (T3, T5) and control and

fertilization treatments of Al-low and No-P. Soil (1-g) was added to a 50-mL centrifuge tube with 0.2 mL toluene, 4 mL modified universal buffer [12.1 g (hydroxymethyl) aminomethane; 11.6 g maleic acid; 14.0 g citric acid; 6.3 g boric acid in 488 mL of 1 N sodium hydroxide (NaOH) and diluted in 1 L of water], and 1 mL of p-nitrophenyl phosphate solution. This mixture was incubated for 1 h before adding 1 mL of 0.5 M CaCl2 and 4 mL of 0.5 M NaOH, then filtered through a Whatman no. 2 filter paper. Samples were measured using a spectrophotometer for yellow color intensity and read at 400 nm (Weaver et al., 1994).

A mixed model factorial analysis of variance (ANOVA; Statistical Analysis Software, version 9.3) for treatment (tomato type, P source, *Trichoderma* isolates and controls, and their interactions) effects on response variables was conducted. Data were evaluated for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) and data were transformed to address non-normal distribution and unequal variances of residual error where appropriate. "Trial" was treated as a random factor. Fisher's protected Least Significant Difference (P-LSD) test was used to separate means at 5% significance level; untransformed means and standard errors are reported. Data were pooled for analysis.

Results

Objective 1. In our first objective, measurements of plant height and stem caliper were collected at harvest (9 weeks post-transplant) but there were no significant differences among treatments. Soil pH was also measured before adding fertilizer and at the end of the study. From random samples, there was an average pH of 6.1 to 6.5 both before and after fertilization. Above ground tissues were analyzed for P mass, tissue P concentration, and plant biomass, with consistent significant effects of tomato type, P treatment, and the interaction of tomato type and phosphorus (Table 3.2). There were significant differences in plant biomass for treatment factors (Table 3.2), including *Trichoderma* ($P = 0.05$). However, all plant biomass (mg/plant) averages were statistically similar within tomato type except for *S. pimpinellifolium,* which showed T3 (5.02 g/plant) had the highest average biomass compared to T5 (3.48 g/plant) and the no *Trichoderma* control (3.91 g/plant). For P mass in tomato tissues (Fig 3.2), K-P was consistently the highest among all tomato types with P mass in *S. l. cerasiforme* (28.7 mg P/plant) being significantly greater than other tomato types, which were all statistically similar (Fig. 3.2). There were significant variations among other P treatments within tomato types. P mass from Al-P high concentration was higher in cerasiforme (15.9 mg P/plant) compared to all other Al-P high and low treatments. However, No-P treatments had the second highest P mass in all other tomatoes. The lowest P mass concentration was in Al-low, *S. habrochaites* (5.30 mg P/plant). For P concentration in tomato (tissue percentage P/dry tissue) showed some differences between tomato type and phosphorus source (Fig. 3.3). *S. cerasiforme* showed greatest P concentration in tissues for Al-high (0.3067 tissue percentage P/dry tissue) and K-P compared to other tomato types. All tomatoes had significant differences between P % in tissues based on P source, with No-P treatments being the lowest among all tomato types.

Table 3.2: Analysis of variance of main effects and interaction effects (tomato type [Tomato], *Trichoderma* strain [*Trichoderma*], phosphorus source and amount [Phosphorus]) on plant tissue phosphorus, plant biomass, and plant tissue phosphorus mass in Objective 1. NS= not significant, $P > 0.1$.

Effect	Tissue P content	Plant biomass	Tissue P/plant
Tomato	<0.0001	<0.0001	<0.0001
Trichoderma	<0.0001	0.05	NS
Phosphorus	<0.0001	<0.0001	<0.0001
Tomato *x* *Trichoderma*	NS	NS	NS
Tomato × Phosphorus	0.0004	0.0006	0.09
***Trichoderma* × Phosphorus**	NS	NS	NS
Tomato × *Trichoderma* × Phosphorus	NS	NS	NS

Table 3.3: Analysis of variance table of main effects and the interaction effect (Trichoderma, phosphorus) on soil inorganic phosphorus in Objective 1 within tomato type. NS= not significant, *P > 0.1*.

Effect	***Solanum habrochaites***	***Solanum pimpinellifolium***	***Solanum lycopersicum* var. *caerasiforme***	**Heirloom**	**Hybrid**
Trichoderma	NS	0.016	NS	0.0169	NS
Phosphorus	<0.0001	NS	<0.0001	<0.0001	NS
***Trichoderma* × Phosphorus**	<0.0001	0.0593	NS	NS	NS

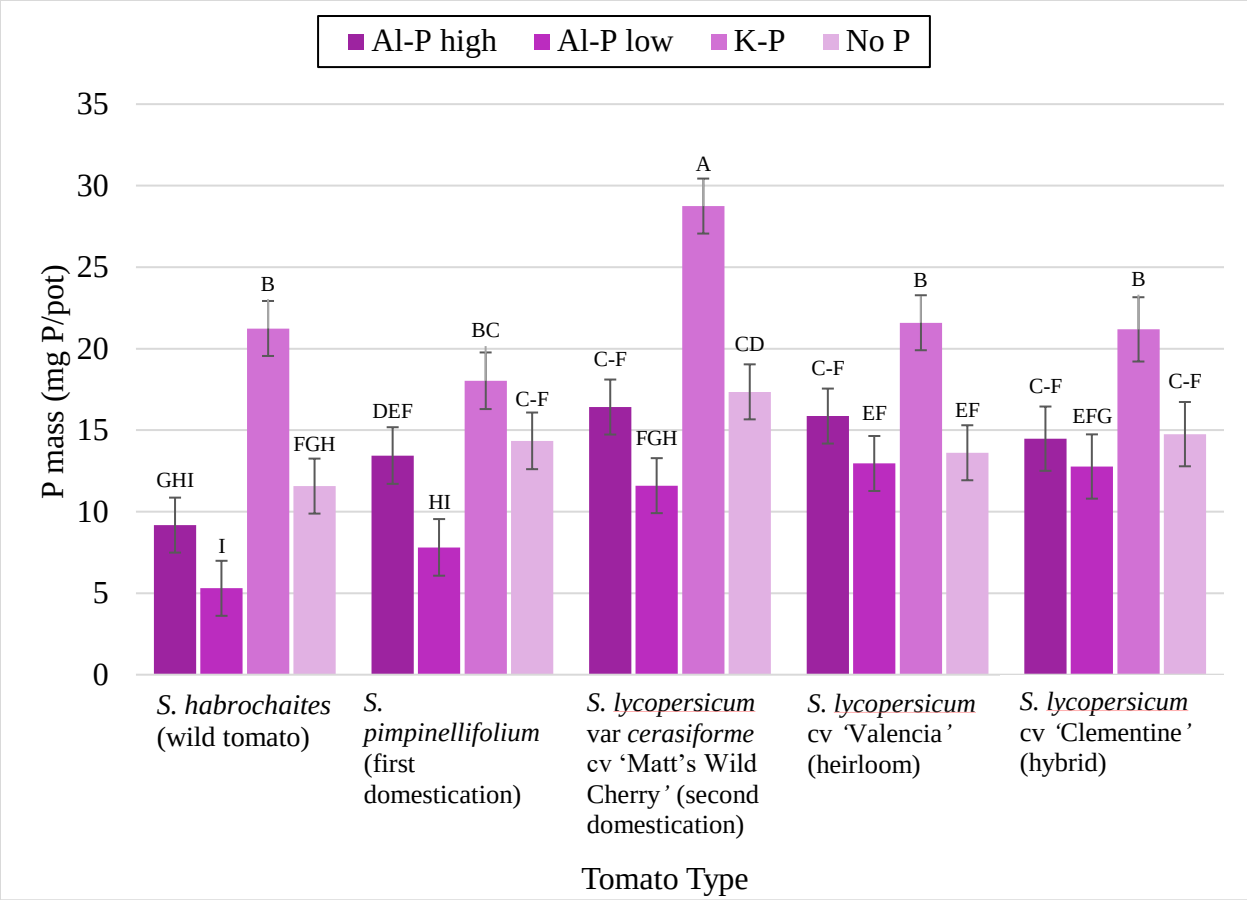

Figure 3.2: P per plant between tomato type and phosphorus treatments. Tomato type is in order of domestication from wild relative (left) to modern/hybrid type (right) in Objective 1. Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

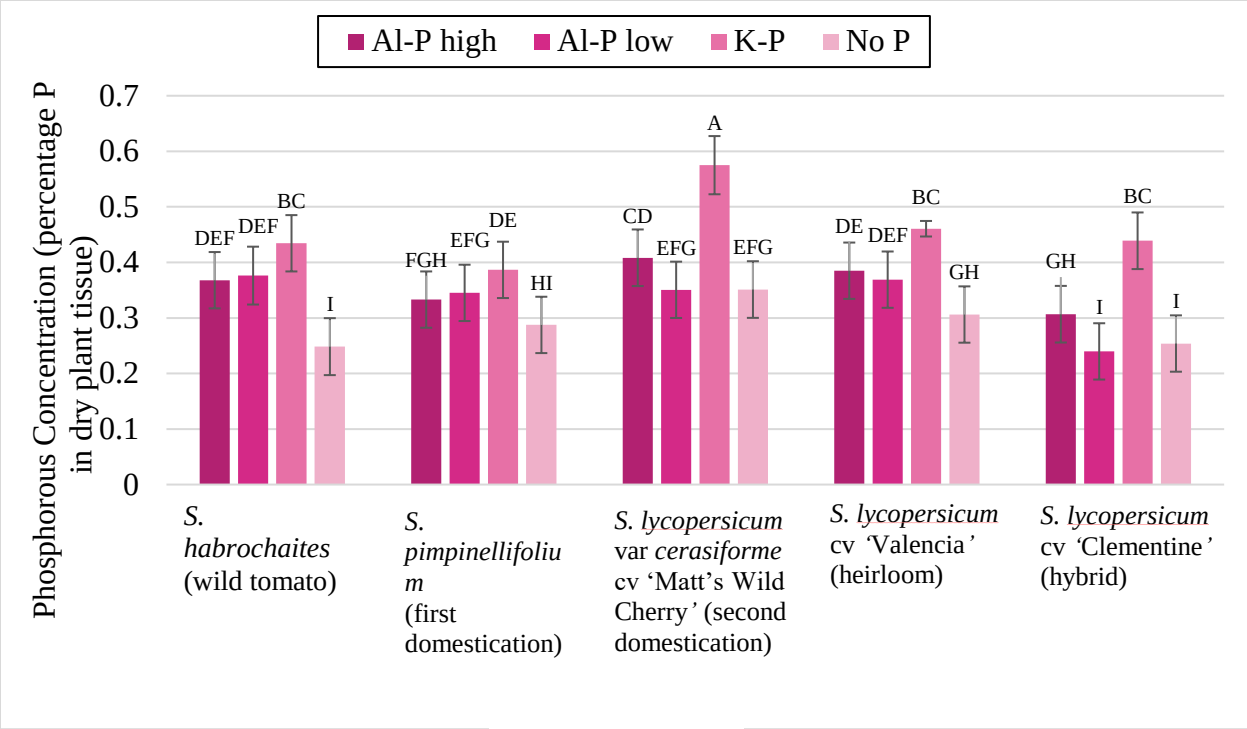

Figure 3.3: Average phosphorus conce[illegible] P in dry plant tissue) between tomato type and phosphorus treatments. Tomato type is in order of domestication from wild relative (left) to modern/hybrid type (right) for Objective 1. Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

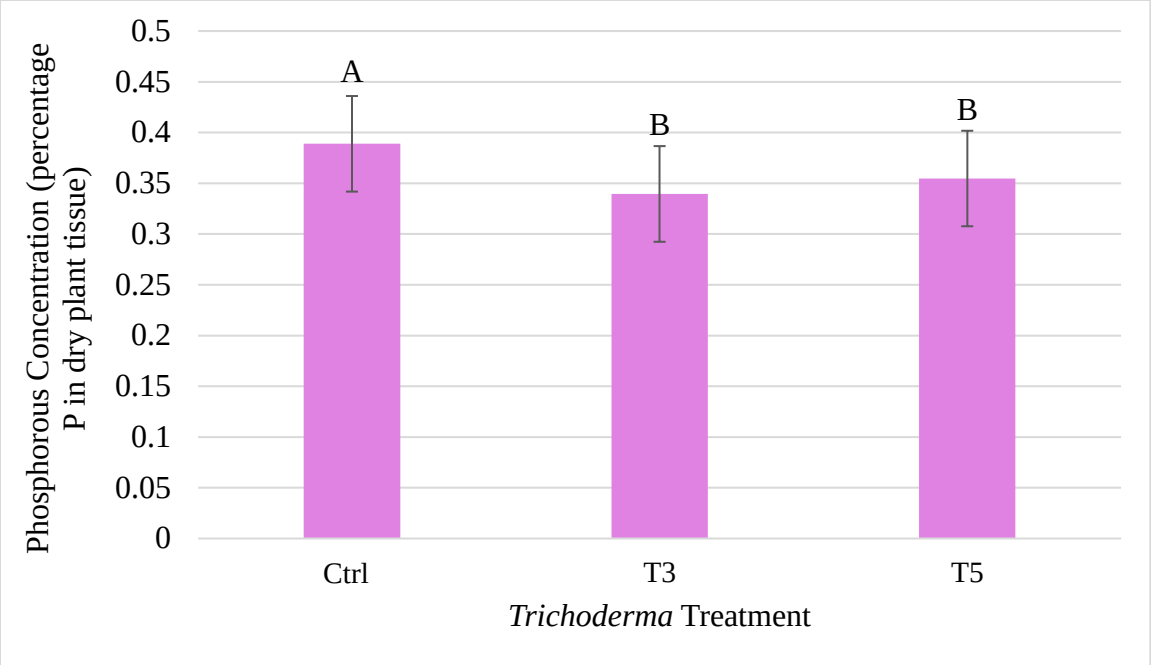

Figure 3.4: Representation of average phosphorus concentration (percentage P in dry plant tissue) between *Trichoderma* treatments T3, T5, and control (no *Trichoderma*) for Objective 1. Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

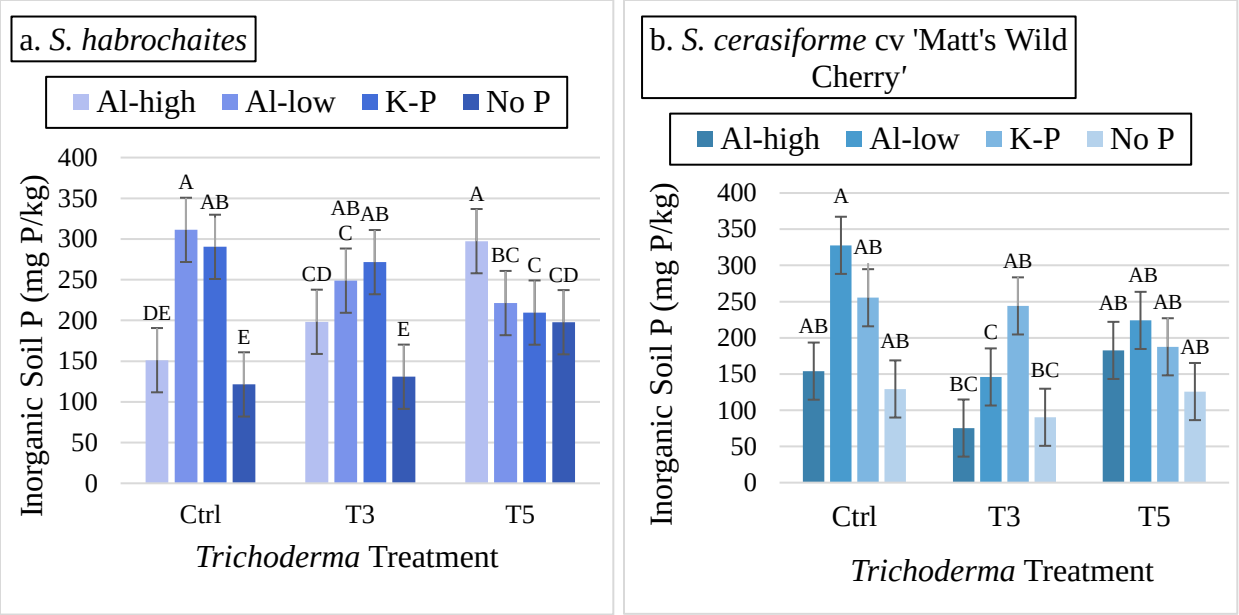

Figure 3.5: Inorganic soil P concentrations (mg P/kg soil) for *habrochaites* (a) and *pimpinellifolium* (b) between *Trichoderma* and phosphorus treatments in Objective 1. Bars denoted with different letters are statistically significant at $P < 0.1$ as determined by Fisher's protected LSD.

The P % in tomato type (tissue percentage P/dry tissue) and *Trichoderma* treatments resulted in control groups (no *Trichoderma*) had highest P concentrations in tissues compared to T5 and T3 treatments, which were statistically similar (Fig. 3.4). In the case of inorganic soil P (Table 3.2), differences were observed in *S. habrochaites* and *S. pimpinellifolium* between P source and *Trichoderma* treatments. For *S. habrochaites* (Fig. 3.5a), control treatments (no *Trichoderma*) had the highest soil P in the Al-low treatment (311mg P/kg soil) soil) while the highest soil P among Al-high (297 mg P/kg soil) was observed from the T5 isolate. For the K-P treatment, soil P concentrations were statistically similar between T3 (271 mg P/kg soil) and control (290 mg P/kg soil) while in the No-P treatments soil P was highest in T5 (197 mg P/kg soil). For *S. pimpinellifolium* treatments (Fig 3.5b), control with Al-low was significantly higher than all other treatments (327 mg P/kg soil) while all other control and T5 treatment groups had no differences. For T3, K-P was like those of T5 and controls, while all other P treatments in T3 were the lowest concentrations of inorganic soil P.

The phosphatase activity was measured between *Trichoderma* treatments, and P source Al-low compared to No-P controls for Valencia (heirloom) tomato (Fig. 3.6). *Trichoderma* control treatments had highest phosphatase activity for Al-low (84.51 mg p-nitrophenol produced/g soil/h) and No-P (70.33 mg p-nitrophenol produced/g soil/h) compared to all other treatment groups. Results of phosphatase activity between *Trichoderma* treatments T3 and T5 were statistically similar.

Objective 2. Inorganic soil P was significantly affected by phosphorus and *Trichoderma* treatments ($P = 0.03$) (Fig. 3.7). The highest P concentration was for the treatment group Al-P/T5 (80.6 mg P/kg soil), while the second largest was Al-P/control (72.9 mg P/kg soil). The No-P treatment groups were statistically similar between T5 (22.6 mg P/kg soil) and control (25.2 mg P/kg soil). There were also significant differences between above ground biomass between tomato types ($P < 0.0001$) (Fig. 3.8). Dry biomass of *S. l. cerasiforme* (48.5 mg/plant) was significantly lower than that of the heirloom (52.1 mg/plant) (Fig. 3.8). There were also significant differences of above ground biomass between phosphorus treatments ($P < 0.0001$) as represented in Fig. 3.8. Dry biomass of No-P (23.9 mg/plant) was significantly lower than that of Al-P (76.7 mg/plant). There was no significant effect of *Trichoderma* treatments, or the three-way interaction (tomato, *Trichoderma*, phosphorus). There were significant differences between disease ratings among tomato types with an interactive effect of *Fol* inoculation (*Fol*/no *Fol*) and P fertilization (Al-P/No-P) ($P = 0.02$). For *S. l. cerasiforme* (Figure 3.9a), both No-P fertilization treatments with and without *Fol* had the highest disease ratings (4) while *Fol* inoculation with Al-P had the lowest disease rating (2.16) compared to no *Fol* with Al-P (3.16). For heirloom tomato (Figure 3.9b) both Al-P fertilization treatments with and without *Fol* had the lowest disease ratings (1.0) compared to *Fol* with No-P (2.33) and no *Fol* with No-P (3.41). There were no significant differences in establishment rates, tomato height, or stem caliper between treatment groups.

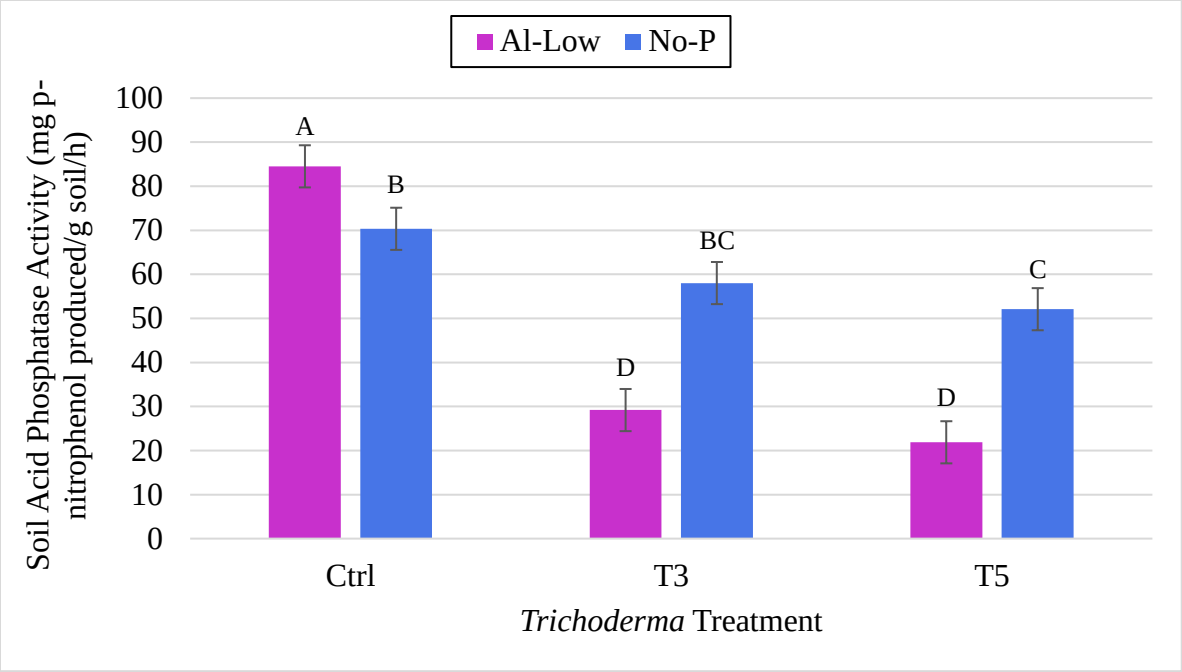

Figure 3.6: Phosphatase activity between *Trichoderma* and control treatments (mg p-nitrophenol produced/g soil/h) for Objective 1, analyzed at 400 nm. Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

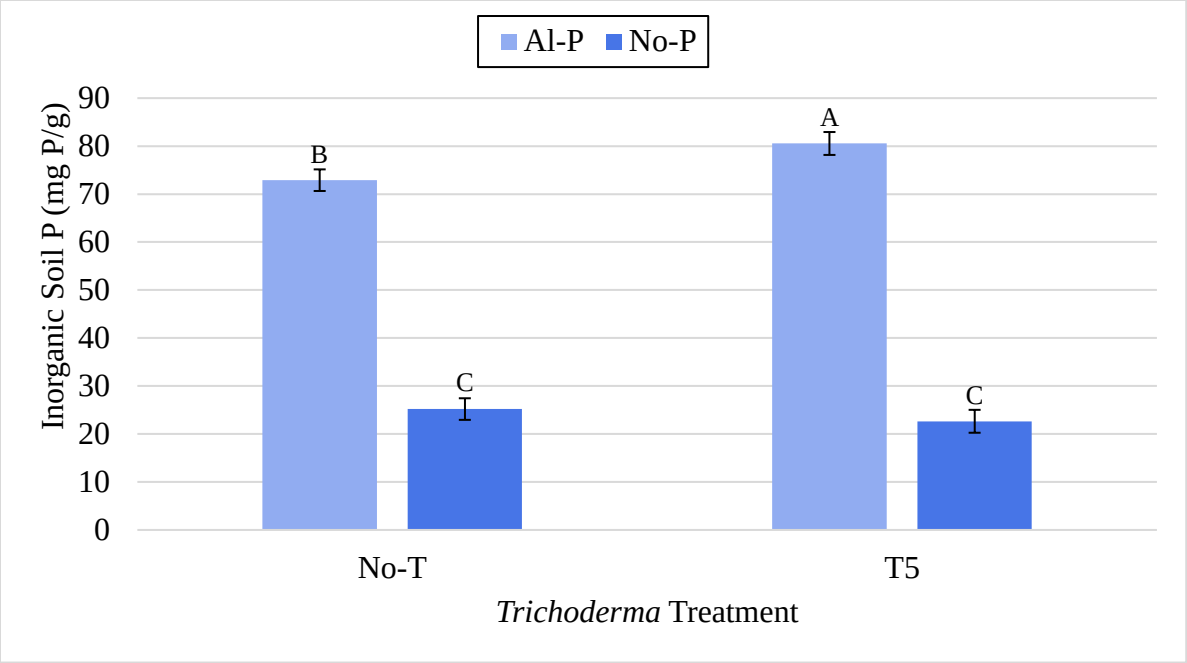

Figure 3.7: Average inorganic P (mg/kg soil) in soil between P treatments with or without *Trichoderma* (T5/ No-T) for Objective 2. Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

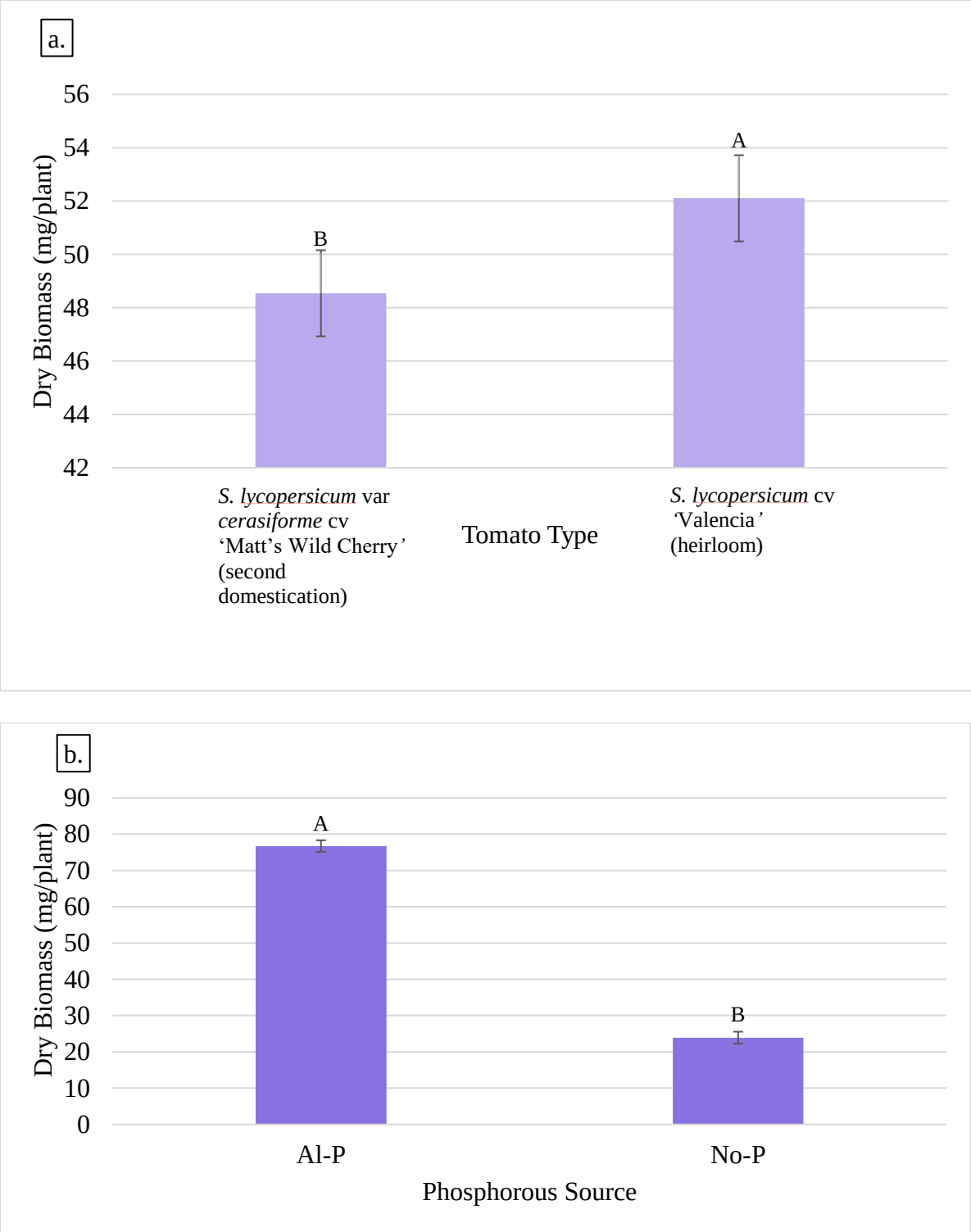

Figure 3.8: Average dry biomass (mg/plant) of above ground tissue between tomato types (a) and between phosphorus treatments (b) for Objective 2. Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

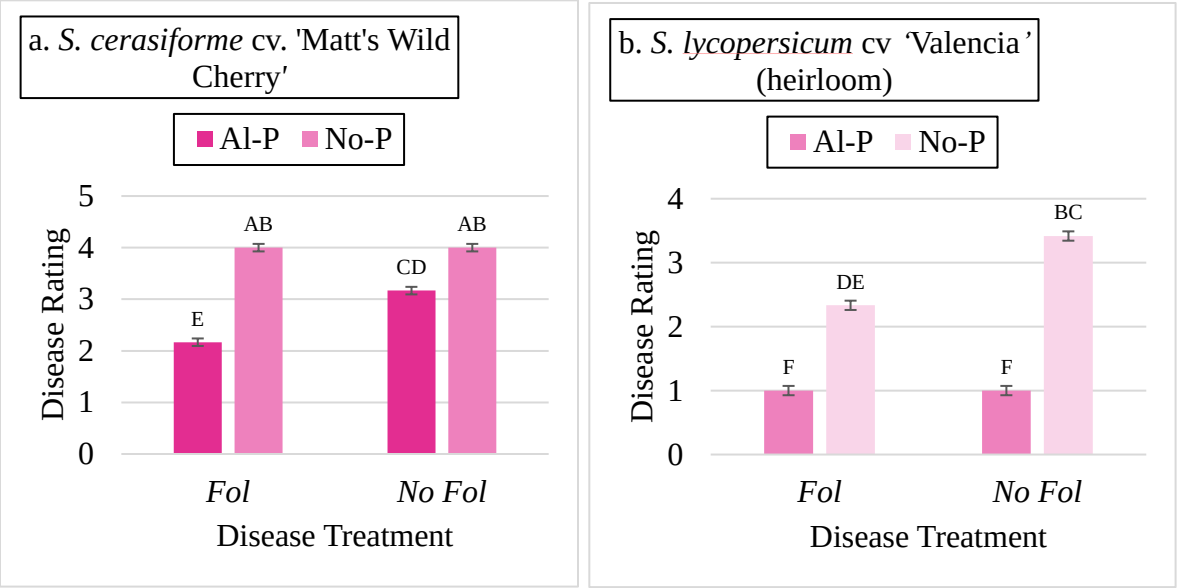

Figure 3.9: Average disease ratings of *Fol* and phosphorus fertilization treatments (Al-P or No-P) for *cerasiforme* (a) and heirloom (b) tomatoes for Objective 2. Bars denoted with different letters are statistically significant at $P < 0.05$ as determined by Fisher's protected LSD.

Root colonization of *Trichoderma* was consistent between treatment groups, as fungal colonies were present throughout the experiment. While there was also evidence of other naturally occurring microbes when root plating, control plants experienced colonization of both *Trichoderma* and *Fol.* In an attempt to determine the impact of potential cross contamination, soil dilutions of control plants were analyzed to determine the CFU for both *Trichoderma* and *Fol*. The rates were < 50 CFU/ g of soil, compared to the application rates of 100,000 CFUs / g of soil (*Fol*) and 1×10^6spores/mL of *Trichoderma,* showing root colonization of other fungi was relatively low.

Discussion and Conclusions

In objective 1, we conducted experiments to better understand the relationships between *Trichoderma,* tomato domestication, and phosphorus uptake with P sources with varying forms for availability in soil environments. We hypothesized that high P-solubilizing *Trichoderma* would increase P uptake when tomato is grown in low-P availability substrates, but the magnitude of effects would differ among tomato germplasm and be more pronounced in tomato wild relatives. Significant differences were observed within results of P mass and percentage P concentrations of above ground plant tissues. Tomato type *S. l. cerasiforme* had the highest concentration of P mass with K-P fertilization, which is considered most readily available compared to Al-P fertilization. While results showed high fertilization rates of Al-P had the greatest P mass in heirloom tomatoes, P mass concentrations of both Al-P fertilization amounts were lower than No-P treatments among all tomato types. We suspect this is due to the limited availability of Al-P in clay soils. Relatively high P uptake from No-P treatments is likely explained by relatively high soil P availability in the field soil used in our experiment. Results of P concentration (mg P/g tissue) in tomato also showed significant ability for tomatoes to uptake Al-P fertilization at the higher application rate. There were also observed differences in tomato biomass, as *S. l. cerasiforme* had the greatest biomass with T3 treatments, compared to T5 and the control. There were significant differences between tomato types, as *S. l. cerasiforme* (second domestication), heirloom, and hybrid types had greater P concentrations in Al-P treatments compared to wild type (*S. habrochaites*) and first domestication (*S. pimpinellifolium)*. These tomatoes showed significant potential for P uptake of Al-P at different fertilization rates compared to other tomatoes, which had no significant differences among treatments. This aids in understanding the benefits of natural genetics in tomato wild types to sustain stressful environmental conditions with limited nutrient availability.

Analysis of inorganic soil P also supported the hypothesis that *Trichoderma* treatments (T3 and T5) show great potential for Al-P solubilization and uptake at different fertilization rates. However, more studies should be conducted to better understand *Trichoderma* mechanisms and potential benefits as a fertilizer enhancer. Soil phosphatase activity was significantly different between *Trichoderma* and control treatments, as phosphatase activity with No-P fertilization was greater than Al-P fertilization groups. This is likely due to other naturally occurring soil microbes working to scavenge for naturally occurring soil organic P from the

organic field soil. In contrast, it is possible that the *Trichoderma* isolates have less phosphatase production within Al-P treatments due to their secretion of organic acids to solubilize Al-P, as organic acids are able to convert soil Al-P into readily available phosphates for plant uptake. However, understanding of enzymatic actives are both complex and limited (Bononi et al., 2020).

In objective 2, we aimed to better understand the biocontrol potential of *Trichoderma* (T5) within genetically diverse tomato when exposed to varying fertilization conditions. As *Fol* can be influenced by fertilization, we aimed to observe differences in tomato resiliency and P uptake within *Trichoderma* treatments against those with *Fol.* Analysis of inorganic soil P showed significant differences between P and *Trichoderma* treatments, with the highest concentration of soil P being present in treatment group Al-P/T5, compared to other treatment groups. The measurements of dry biomass of plant tissue resulted in the greatest plant growth in those treated with Al-P compared to No-P, suggesting that tomatoes did have nutrient benefits from Al-P treatments, even though it is not a readily available source of P. Benefits of Al-P fertilization were also supported within results of disease ratings, as treatments with Al-P had lower disease ratings and overall stress symptoms compared to those with No-P. Disease ratings were higher in *S. l. cerasiforme* compared to heirloom tomato treatments. This result also corresponds with the average dry biomass of tomatoes, as *S. l. cersiforme* was significantly lower than heirloom tomato.

Reflecting on both objectives, results of tomato biomass for P concentration, P mass, and plant biomass help to support the idea that tomato domestication has a direct effect on P uptake efficiency, while some tomatoes can also vary in ability to benefit from *Trichoderma* interactions, such as those for increased plant growth and P availability. These results support the conclusions of Dixon et al. (2020), where authors reported a 77% increase in tomato dry weight with P efficient tomato groups. Previous findings of Banani (2014) and Jaiswal (2020) and Smulders (2020) also concluded that genetics and growth habits can vary within tomato types, especially wild tomatoes compared to modern heirloom and hybrids. We also observed the influence of tomato genetics for *Fol* resiliency, as our tomatoes varied significantly in *Fol* disease symptoms.

While this study had significant results to aid in understanding of the relationships between *Trichoderma*, P fertilization, and tomato domestication, studies should be continued to better understand complex mechanisms of *Trichoderma* solubilization of P in varying forms, especially for improving plant availability, along with the influence of P for *Fol* disease severity. Future studies should also aim to analyze more *Trichoderma* isolates to aid in development of commercial products for plant growth promoters and fertilizer enhancers to benefit crop production settings, especially for tomato.

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VITA

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