Considering the rhizosphere in plant tolerance to herbivory among wild and domesticated tomatoes

OVERVIEW

As the root microbiome's role in plant defenses against insect herbivores becomes clearer, scientific focus has lingered on a single side of plant defense: resistance. Its indispensable counterpart, tolerance, is comparatively overlooked in such investigations, despite its immense value as an evolutionarily sustainable mitigator of herbivore damage. This thesis seeks to expand our limited understanding of the extent to which tolerance to herbivory may by influenced by rhizosphere microbial communities. Specifically, I aim to (1) determine if rhizosphere communities contribute to expressed tolerance (i.e. if tolerance has a hologenomic basis) and (2) investigate the relationship between rhizosphere community characteristics and tolerance to herbivory. In an agroecosystem setting, I will expose seven tomato lines to herbivory by the specialist tobacco hornworm (*Manduca sexta*). Collected fruits and rhizosphere samples will be used to estimate tolerance and characterize rhizosphere communities, respectively. In a second greenhouse experiment, I will again compare the tolerance of two tomato lines to hornworm herbivory, but involve a sterilized soil treatment to explore if the presence of root microbiome affects tolerance. This research will shed much needed light on what role, if any, the rhizosphere plays in plant tolerance to herbivory. Ultimately, this will point to avenues through which plant defenses could be amended in pest management and complement our understanding of the interactions between host plants and their specialist herbivores.

INTRODUCTION

Plant defenses against insect herbivory

In response to herbivory, plants rely on a blend of defensive mechanisms in efforts to mitigate fitness losses and deter herbivore feeding. The latter is achieved through resistance traits, which curtail herbivory by reducing herbivore fitness (antibiosis) or affecting herbivore behavior such that it less successfully colonizes a plant (antixenosis) (Painter 1951, Kogan and Ortman 1978). An aphid, for example, may grapple with both types of resistance on a host plant that stunts population growth rates and offers unpalatable phloem that deters winged aphid settlement (Klingler 2005). Though resistance mechanisms effectively combat resource loss, they can also lead to cycles of escalating defenses on macroevolutionary scales; as insects acquire resistance to plant metabolites, selection favors plants that create increasingly more potent and ultimately costly defensive weaponry (Dawkins and Krebs 1979).

Not all plant defenses engage these arms races, however; resistance's compatriot, tolerance, is thought to bring about fewer coevolutionary consequences, since tolerance mechanisms target intrinsic plant processes rather than engaging herbivore responses (Mauricio 2000, but see Fornoni 2011). Targeted plant processes involve mobilizing nutrients around sites of herbivore feeding, maximizing metabolic efficiency, and mitigating oxidative stress (Strauss and Agrawal 1999, Koch et al. 2016). Through these mechanisms, tolerance traits ultimately seek to minimize plant fitness losses during herbivory (Stowe et al. 2000).

Expressing tolerance calls upon both roots and shoots. Aboveground, plants can increase photosynthetic rates to recoup resources lost to herbivores (Nowak and Caldwell 1984) or invest in fewer, smaller leaves to maximize canopy light penetrance and, in turn, increase photosynthetic efficiency (Augustine et al. 2011). Roots, on the other hand, play critical roles in nutrient storage and mobilization. These storage organs temporarily sequester nutrients from aboveground herbivores (Schwachtje et al. 2006) and later shunt these resources aboveground to fuel compensatory growth after the herbivory event has ended (McNaughton 1983).

Roots play another critical role, however, that is rarely discussed alongside plant tolerance; roots actively release photoassimilates, mucilage, secondary metabolites, and other exudates into the rhizosphere (Bais et al. 2006, Loyola-Vargas et al. 2007), creating a nutrient-rich zone that attracts an overwhelming diversity of soil microbes (Schenck zu Schweinsberg-Mickan et al. 2012). The resultant microbial community, called the root microbiome or rhizosphere, interacts with the host plant to influence all aspects of plant physiology, including defense (Bulgarelli et al. 2013). We have a foundational awareness of how the rhizosphere contributes to resistance, notably by priming host plant defenses (Pieterse et al. 2014). However, our understanding of how this community affects plant tolerance to herbivory is comparatively lacking and compounded by uncertainties surrounding the genetic and mechanistic basis behind how plants tolerate herbivory (Stowe et al. 2000, Fornoni 2011, Peterson et al. 2017). Potential for a microbial hand in tolerance mechanisms exists in abundance but evidence remains scattered in the literature and rarely discussed within a framework of plant tolerance. This leaves us with a piecemeal understanding of how the root microbiome may influence a plant's ability to tolerate herbivory. My research aims to address this gap by examining rhizosphere community characteristics associated with tolerant traits.

The role of roots in plant tolerance

As previously mentioned, roots are more than just organs for nutrient acquisition and excretion. Roots are also critical in resisting and tolerating herbivory, even when damage occurs in a plant's aboveground tissues. These organs can act as synthesis sites for anti-herbivore secondary metabolites fated for leaves, reservoirs for carbon sequestered from aboveground herbivore grasp, and recruitment centers for microbiota that influence plant health and defenses (Nalam and Nachappa 2014). The latter two roles have direct implications for a plant's ability to tolerate herbivory.

Investment in belowground biomass prior to herbivore damage can increase plant tolerance, as these robust carbon and nitrogen stores insure against resource losses to herbivores and increase absorption of soil nutrients and water (Hochwender et al. 2000). Roots can also act as more dynamic storage organs by temporarily storing nutrients shunted from shoots in response to aboveground herbivory (Orians et al. 2011). By mobilizing nutrients only in response to species-specific herbivore elicitors (Halitschke et al. 2003, Schwachtje et al. 2006), plants tailor this response to herbivores for which tolerance may prove more effective, such as specialists (Orians et al. 2011, Steinbrenner 2011). This act of induced resource sequestration safeguards nutrients from aboveground herbivore consumption, though it comes with ecological costs; while carbon is sequestered, plants experience slower growth (Orians et al. 2011) and resource vulnerability to belowground herbivores (Kaplan et

al. 2008). However, once herbivore pressure lessens, the plant can reallocate sequestered resources aboveground for repair, regrowth, and reproduction. In this way, carbon sequestration can minimize fitness losses to aboveground herbivores and may even increase overall plant fitness by prolonging senescence and increasing reproductive output (Schwachtje et al. 2006, Garcia and Eubanks 2019).

Along with resource sequestration, many plants increase short-term root exudation in response to insect feeding (Hamilton and Frank 2001, Fu and Cheng 2004, Henry et al. 2008), with exudation volume reflecting herbivory intensity (Holland 1996). This nutrient pulse supports increased root microbial biomass (Fu and Cheng 2004, Henry et al. 2008), and these microbial communities can turn over more bioavailable nitrogen (Clarholm 1985, Hamilton and Frank 2001). Enhanced nutrient absorption could then bolster plant investments in post-herbivory compensatory growth. Exudation volume falls off a few days after the herbivory event ends (Henry et al. 2008), so these exudation surges could be part of plant efforts to temporarily but rapidly recruit the microbial communities that can best ease them through major biotic stresses.

Most studies of herbivory-induced exudation have strictly considered grasses and chewing herbivores. More research is sorely needed to explore if other plant types and insect feeding guilds engage in the same patterns of herbivore-induced exudation. In addition, though many report increased root microbial biomass in response to short-term exudation surges, the benefit, if any, of this robust rhizosphere community is unclear beyond an increase in plant nitrogen absorption (Hamilton and Frank 2001). Considering the demonstrated importance of the rhizosphere community in resistance to herbivory, as well as the potential for a microbial hand in tolerance, this community could be instrumental in supplementing plant defenses.

Potential for microbially-mediated tolerance

Plant traits alone do not determine defense responses; microbial members of the rhizosphere also play a nuanced and powerful role in plant defense. Evidence for their hand in resistance has been well-documented (Berendsen et al. 2012) and most notably includes microbial priming of plant defenses in a process called induced systemic resistance (Pieterse et al. 2014). Rhizosphere communities are also instrumental in retarding insect growth (Valenzuela-Soto et al. 2010), augmenting secondary metabolite production (Lugtenberg and Kamilova 2009, Shoresh et al. 2010, Matilla and Krell 2018), and altering or supplementing plant volatiles to attract herbivore natural enemies (Guerrieri et al. 2004, Hempel et al. 2009).

Despite this ample evidence for microbially-mediated mechanisms of plant resistance, we know little about the microbial underpinnings of plant tolerance to herbivory (Vannette and Hunter 2009). One avenue through which microbes may influence plant tolerance is in the amendment of plant nutrient uptake. Evidence of rhizosphere communities supplementing plant nutrition are ample (Bulgarelli et al. 2013). For example, after herbivore-induced surges in root exudation, simple exudates consumed by nitrogen mineralizing bacteria support populations of predatory protozoa that create an excess of bioavailable nitrogen (Clarholm 1985, Hamilton and Frank 2001). Nutrient supplementing PGPM would be a logical boon to tolerance efforts to establish robust resource reserves before and recover lost tissues after herbivory. However, plants amended with these nutrition supplementing PGPM can also become more desirable food sources owing to increases in

leaf amino acid content (Badri et al. 2013). These amended plants consequently experience more severe herbivory from chewers (Badri et al. 2013) but particularly from sap feeding insects (Gange and West 1994). This trend underscores the importance of studying these PGPM and, even better, microbiomes, explicitly under the context of tolerant responses to insect herbivory.

In investigating the roles of this rhizosphere community in plant defense, some taxa have been given more consideration than others. Specifically, most research on plant-microbe interactions strictly considers the role of rhizobacteria and, less often, arbuscular mycorrhizal fungi. Other taxa have unexplored potential for a hand in microbially mediated defense. Saprotrophic fungi, for example, are an expansive functional group that contains plant pathogens which act as facultative saprotrophs en route to plant roots (e.g. pathogenic strains of *Fusarium oxysporum*), a handful of well-known mutualists (e.g. *Trichoderma* spp.), and many more understudied decomposers (Joergensen 2000). Compared to bacteria, fungi typically excel at decomposing complex substrates (Rousk and Bååth 2011), such as the mucilage and polysaccharides exuded in large quantities by roots (Bais et al. 2006). The group may create a more productive microenvironment by providing primary or supplemental nutrition for bacteria, many of which are able to attach to and feed on fungal hyphae (Rudnick et al. 2015), or by extending hyphae further into the soil matrix to access distant nutrient sources and connect habitats for less motile mycophagous microbes (Ballhausen et al. 2016). These fungal saprotrophs should be given more consideration in future investigations of the tolerant root microbiome.

Tolerance-conferring microbiomes may also play roles in the many other mechanisms of plant tolerance. Evidence for microbes filling these roles exists, but what literature addresses this potential usually focuses on single strains, rarely considering their place as one of many members in the rhizosphere. Single-strain investigations have offered evidence for microbial species which are associated with post-herbivory increased plant photosynthetic rates (*Arabidopsis*, Zhang et al. 2008; *Solanum*, Valenzuela-Soto et al. 2010), stimulated regrowth of roots and shoots (*Oryza*, Cosme et al. 2016), antioxidant synthesis (*Abelmoschus,* Habib et al. 2016; *Hordeum*, Waller et al. 2005, Baltruschat et al. 2008) and ethylene regulation (Glick et al. 2007) to combat oxidative stress, and improved yields (*Capsicum*, Herman et al. 2008), all of which are often cited as tolerance traits (Strauss and Agrawal 1999, Koch et al. 2016). Evidently, opportunities for root microbes to shape plant tolerance are ample, but we have little idea what a tolerant rhizosphere may look like on the whole-community level. Studies exploring this community are needed if we are to understand how a plant, as a holobiont, tolerates insect herbivory and how microbial communities may be leveraged to buoy plants through biotic stresses.

Consequences of crop domestication for microbial components of plant defense

Plants have evolved to best tailor their root microenvironments to target beneficial microbes (Brundrett 2002). This is accomplished through the exudation of metabolites that target specific microbial communities (Bais et al. 2006). This coevolutionary functionality can be disrupted, however, in the case of extreme selection events such as domestication. The targeted selection and population bottlenecks that accompany domestication have unpredictable and often detrimental impacts on plant defensive physiology (Whitehead et al. 2017).

Domestication has largely increased crop susceptibility to insect herbivores, with insects faring better on and preferring crops compared to their wild relatives (Chen et al. 2015, Whitehead et al. 2017). This could be explained by their enhanced nutritional status (Roucou et al. 2017) or reduced defenses; secondary metabolite production appears to have decreased over the domestication process (Meyer et al. 2012, Chen et al. 2015), but some have specified that such an association exists only in the plant's harvestable organs (Whitehead et al. 2017).

More often than not, the harvestable organs on which human selection has focused are produced aboveground. In these cases, crops typically allocate less biomass belowground, producing fewer and more shallow roots compared to their wild relatives (Pérez-Jaramillo et al. 2016, Roucou et al. 2017). These traits impact a plant's access to and ability to acquire resources from surrounding soil, and generally decreases crop tolerance to herbivory compared to that of its wild relatives (Welter and Stegall 1993). In addition, domestication may bring about unpredictable shifts in root exudation. Changes to both root architecture and exudation likely hamper a crop's ability to recruit and retain in sufficient abundance the microbial communities from which their wild relatives benefit (Pérez-Jaramillo et al. 2016).

In general, the root microbiomes of crops appear to be less diverse than those of their wild relatives (Zachow et al. 2014), which is potentially explained by the differences in metabolites exuded by domesticated and wild plants (Ianucci et al. 2017). There are also broad taxonomic trends among wild and domesticated plants, with crop roots typically housing more Actinobacteria and Proteobacteria (Pérez-Jaramillo et al. 2018), and wild relatives fostering a more robust community of Bacteroidetes. The latter taxon is often described in the context of its ability to degrade complex biopolymers (Thomas et al. 2011), and Bacteroidetes are relatively abundant in the rhizospheres of mature crops which exude more complex root exudates (Chaparro et al. 2014). This would seem to signal a decline in the abundance of complex exudates, including secondary metabolites, secreted by domesticated cultivars. These trends, though general, suggest that domestication-derived changes in root traits have extensive consequences for crop microbial communities. At present, most discrepancies between the tolerance of wild and domesticated plants are attributed to altered root architecture or canopy structure (Welter and Stegall 1993, Roucou et al. 2017). The potential for rhizosphere communities to contribute to this discrepancy in need of future attention (Pérez-Jaramillo et al. 2016).

Tomato: a model crop for investigating microbially mediated defense against herbivory

Tomatoes make up an excellent system with which to ask questions about the microbial underpinnings of plant tolerance to herbivory. Members of Solanaceae are known for their exceptional tolerance to herbivory. Tomato use of tolerance mechanisms such as root carbon sequestration and compensatory growth is well documented (Steinbrenner et al. 2011, Gómez et al. 2012, Korpita et al. 2014). These mechanisms are especially relied upon in response to specialist herbivory from the voracious tobacco hornworm (*Manduca sexta*). This model organism is well-studied, and tomatoes uniquely respond to hornworm herbivory with nutrient mobilizing tolerant responses, while relying on resistant metabolites in response to generalist herbivory (Steinbrenner et

al. 2011) Tomatoes have even been documented overcompensating for herbivory when fruits were damaged by tomato bugs (Sánchez and Lacasa 2008).

Though domesticated tomatoes possess relatively exceptional tolerance to herbivory, they appear to have lost much of their potential for tolerant responses over the domestication process; wild relatives are more tolerant to defoliation than domesticated cultivars (Welter and Steggall 1993, Carrillo et al. 2019, Paudel et al. 2019). Previously, this has been explained by wild relatives' constitutively robust carbon stores, larger root:shoot ratios, (Welter and Steggall 1993, Carrillo et al. 2019, Paudel et al. 2019), and efficient canopy structure. However, the role of the rhizosphere community in determining tolerant responses to herbivory remains unconsidered. Root microbial communities of wild and domesticated tomatoes are unique (Carrillo et al. 2019, French et al. 2020). There exists ample potential for root microbial influence over plant tolerance, and considering the spectrum of tolerance in wild and domesticated tomatoes alongside their unique root microbiomes, this system is ideal to pursue investigations into the extent of rhizosphere involvement in modulating plant tolerance to herbivory.

RATIONALE AND SIGNIFICANCE

In recent examinations of the microbial underpinnings of plant defenses, tolerance has been overlooked in favor of resistance. Much needed attention to microbial involvement in this component of plant defense would offer opportunities to support evolutionarily sustainable traits that could buoy crops through herbivore damage in pest management practices (Peterson et al. 2018). Many modern plant breeding efforts and pest management tactics, including, for example, the integration of Bt traits, seek to change herbivore biology or behavior in order to minimize feeding damage. These practices place pressure on insect populations to develop traits that resist the plant's defensive strategies (Peterson et al. 2018). Tolerance, on the other hand, does not bait the development of resistant populations; this is because its mechanisms target intrinsic plant traits that allow plants to minimize and recuperate losses, such as photosynthetic rates, rather than targeting insect traits (Mauricio 2000, but see Fornoni 2011 for a more nuanced discussion of how plant tolerance may impose selection pressures on herbivores).

If we understand the qualities of a tolerance-conferring root microbiome, we can both breed plants to foster suitable microenvironments for these beneficial microbiomes and develop microbial amendments to increase crop tolerance to herbivore damage. These genetic and microbial additions would represent relatively stable pest management options, as they would not encourage the development of insect populations that thwarted those tolerance traits. The research proposed here represents a fundamental step in this direction by (1) establishing if the presence of a rhizosphere influences tolerance and (2) identifying rhizosphere community characteristics associated with tolerance to herbivory.

OBJECTIVES

This research aims to improve our understanding of how rhizosphere communities influence plant tolerance to insect herbivory. I propose to accomplish this by first characterizing the tolerance and rhizosphere communities of tobacco hornworm-infested and -uninfested tomato lines running across a domestication gradient (Chapter 1). I will then examine if rhizosphere communities contribute to the expression of tolerance (i.e. if tolerance has a hologenomic basis) by comparing plant tolerance among tomato lines grown with and without a root microbiome (Chapter 2). This proposal consists of two objectives:

Objective 1.1: Compare the tolerance of wild and domesticated tomatoes to specialist herbivory.

H_A: Tomato wild relatives are more tolerant to herbivory than domesticated cultivars. Using yield as a proxy for tolerance, this hypothesis predicts that yield differences between infested and uninfested plants will be greater in domesticated cultivars compared to wild relatives.

H_0: No significant difference in tolerance to herbivory will exist among wild and domesticated tomatoes.

Objective 1.2: Characterize and compare rhizosphere communities across plant growth stage, a domestication gradient, and levels of herbivore infestation.

$H_{(Time)}$: Tomato line rhizospheres will be unique in early season samples, but become obscured in late season samples (i.e. there will be a significant difference between rhizosphere communities of unique lines in the early season samples, but communities will no longer be significantly different in late season samples).

$H_{(Domestication)}$: Rhizosphere communities will differ significantly between tomato lines, with the rhizospheres of wild relatives grouping more closely with other wild relatives than with domesticated cultivars. Rhizosphere communities of wild relatives will also be more diverse than those of domesticated tomatoes. This hypothesis is based on the assumption that domestication disrupts beneficial tomato-rhizosphere interactions and, consequently, domesticated tomatoes have lost the ability to recruit and retain specific suites of beneficial microbes. Taxa predictions?

$H_{(Herbivory)}$: Rhizosphere communities of plants experiencing herbivory treatments will show enrichment of a unique set of microbial taxa compared to those of uninfested plants. Microbial biomass will be higher in plants experiencing herbivory, partially owing to anticipated increases in root exudation, though this will not be tested.

H_0: Rhizosphere communities will not significantly differ between early- and mid-season sampling dates, domestication extent, or herbivory treatments.

Objective 1.3: Investigate the relationship between rhizosphere community characteristics and plant tolerance to herbivory.

H_A: All of the following will be significantly correlated with plant tolerance to herbivory: microbial biomass, diversity, [taxa predictions]

H_0: No rhizosphere community characteristics or taxa will significantly correlate with tolerance to herbivory.

Objective 2: Examine the extent to which the tomato rhizosphere mediates tolerance to herbivory

H_A: Plants lacking a root microbiome will have significantly lower tolerance to herbivory (i.e. yield differences between infested and uninfested plants will be significantly greater when plants are grown in sterile soil compared to undisturbed soil).

H_0: There will be no significant difference in tolerance between plants with and without a root microbiome.

APPROACH AND METHODOLOGY

Methods (Obj. 1): Characterize tolerance and rhizosphere communities of tobacco hornworm-infested and -uninfested tomato lines across a domestication gradient

In order to examine the tolerance of wild and domesticated tomatoes to defoliation, as well as their associated rhizosphere communities, I will grow seven tomato lines spanning a spectrum of domestication. Half of all plants from each line will be extensively (50%) defoliated by tobacco hornworms (*Manduca sexta*) over a two week period before fruit set. Immediately after defoliation, and once again at the end of the growing season, I will collect rhizosphere samples from each plant. Throughout the growing season, I will harvest ripe tomatoes to collect a proxy for plant tolerance. This will allow me to later correlate plant tolerance with rhizosphere community characteristics to describe a rhizosphere that enhances or limits plant tolerance to herbivory.

The seven selected tomato lines consist of two domesticated cultivars: *Solanum lycopersicum* cv. 'Better Boy' and *S. lycopersicum* cv. 'Sioux;' one semi-wild cultivar: *Solanum lycopersicum* var. cerasiforme, 'Matt's Wild Cherry;' and four wild relatives: *Solanum pimpinellifolium acc. X, S. peruvianum* acc. LA3640*, S. chmielewskii* acc. 1306 and *S. chilense* acc. LA0470. Wild seeds will be sown ten days earlier (28 April 2020) than domesticated seeds (8 May 2020) in order to accommodate the slower growth rate of wild species in preparation for field transplanting. All seeds will be surface sterilized and started in 6x12 seedling trays, then kept in a greenhouse at for six weeks. Seedlings will be allowed to harden off in a shadehouse for a week, then hand transplanted to in a field at the Meigs Horticultural Farm at Throckmorton Purdue Agricultural Center in Lafayette, IN, USA. The field will contain ten rows of 60 plants with the twelve treatments (tomato line (n=6) X infestation (n=2)) organized in 50 blocks in a randomized complete block design. Failed transplants will be replaced as necessary for the week following. Plants will be allowed to grow for four weeks, after which infestation treatments will begin.

Tobacco hornworm infestation

In order to defoliate infested plants, I will use one- to two-week-old tobacco hornworms. Defoliation to 50% was chosen because differences in tomato fruit number and weight are only apparent at >30% defoliation (Walter and Steggall 1993). Hornworm eggs will be purchased from Great Lakes Hornworms and, upon eclosion, hornworms will be raised on an artificial diet for a week at room temperature.

Domesticated tomatoes typically grower quicker and produce fewer leaves than wild tomatoes (Carrillo et al. 2019), so it is important to calibrate hornworm infestation to each plant's approximate size. In early June, each plant's true leaves will be counted. Over the following two weeks, insect nets, each containing two hornworm larvae, will be placed on true leaves toward the bottom of the plant, excluding senescing leaves. Nets will be secured with zip ties. Most infested plants will receive two bags and four hornworms each. Plants with fewer true leaves will receive one bag (two hornworm larvae). Hornworm larvae will be allowed to feed until the bagged leaf is entirely consumed, after which they will be moved to a leaf above and opposite the previous leaf. The timing of this infestation mirrors the natural first wave tobacco hornworm larvae in the Midwest region (Thurston 1965).

It should be noted that insect nets allow supplementary herbivory by wild tobacco hornworms and other insect pests. When comparing domesticated crops and wild ancestors, insect pests generally prefer to feed, aggregate, and oviposit on domesticated cultivars compared to wild relatives (Chen et al. 2015). Therefore, domesticated crops may experience more intense herbivore pressure as a result of local pest preference and success. Recording the presence of other insect pests, as well as signs of any nutritional stress or disease, may be helpful in capturing some of this potential variation. These surveys will be completed with rhizosphere harvests and will describe the presence of non-hornworm pests and approximate uncontrolled defoliation caused by wild hornworm populations. Pests such as Colorado potato beetles or wild tobacco hornworms will be removed from tomato plants if found.

Rhizosphere harvests

At two points during the field season, tomato plants will be removed from the field for rhizosphere harvests. The first sampling period will occur one week after 50% defoliation has been instated and fifteen of the fifty blocks will be removed for sampling. I will uproot plants, remove visible roots from the soil and plant with sterilized scissors, taking care to remove any clumps of soil, and place these roots in a plastic bag. To dislodge surface soil, I will then shake and massage these loose roots. Once roots are removed from the bag, I will collect three 0.5ml samples of this rhizosphere soil from each plant in three sterilized 1.5ml Eppendorf tube and temporarily store samples in a cooler. Upon returning from the field, these samples will be stored at -80C.

After rhizosphere samples have been collected, I will use the same plastic bag to collect at least one cup of soil from the area around the plant. Bulk soil samples will be stored at -30C until they can be shipped to A&L Great Lakes Laboratories for chemical analysis, including organic matter, phosphorus, potassium magnesium, calcium, and pH estimates.

Rhizosphere samples will be used to (1) estimate overall microbial biomass through qPCR, (2) perform community profiling metabarcoding with 16s/ITS primers, and (3) estimate biomass of saprotrophic fungi. The latter estimation can be accomplished by quantifying ergosterol, a cell membrane sterol found in saprotrophic and ectomycorrhizal fungi (but crucially absent from arbuscular mycorrhizal fungi) (Weete et al. 2010). Ectomycorrhizal fungi do not associate with agricultural crops, and therefore I would not expect to encounter it while sampling agricultural soil.

The second rhizosphere harvest will occur four weeks after 80% of a given tomato line's plants set fruit or immediately before frost if the latter comes first. This will force rhizosphere samples to occur at different points in the growing season, at which abiotic factors such as rainfall can influence rhizosphere communities. However, this method samples rhizospheres of plants of comparable maturity, which has an extensive impact on rhizosphere community composition (İnceoğlu et al. 2011, Chaparro et al. 2014). In this harvest, 25 of the remaining 35 blocks will be removed, leaving the remaining ten blocks for fruit harvest collection and prolonged senescence observations.

Quantifying Tolerance to Herbivory

Once fruits ripen, I will begin weekly harvest of ripe fruits on all plants, recording their number and mass (in grams). Harvest data will be used as a proxy for tolerance in future analyses (see Objective 2). Tomato lines for which weekly average yield differences between infestation treatments is largest will be deemed high tolerance lines. As previously mentioned, ten blocks will remain throughout the growing season to monitor changes in fruit yield and evidence for prolonged senescence, which is a tolerance mechanism that has been documented to increase fitness in response to herbivory in tobacco, a solanaceous relative of tomato (Schwachtje et al. 2006).

Methods (Obj. 2): Examine the extent to which the tomato rhizosphere mediates tolerance to herbivory

Objective 1 explores rhizosphere communities associated with plant tolerance in realistic agroecosystem environments; however, it does not eliminate the possibility that observed differences in tolerance could be entirely explained by intrinsic plant traits that vary between lines, such as root architecture, rather than each line's rhizosphere community. In order to address this limitation, I propose comparing tolerance among tomato lines with and without a root microbiome in a greenhouse setting.

First, I will identify high and low tolerance lines from the seven tomato lines considered in Objective 1. These will be selected by examining yield data from Objective 1. The line for which weekly average yield differences between infestation treatments (uninfested yield – infested yield) is largest will be selected as the low tolerance line. The line for which yield differences between treatments is closest to zero or, in the case of overcompensation, negative, will be selected as the high tolerance line. We may also choose to select a line of intermediate tolerance using the same method if there is such a clear intermediate candidate. If no such clear candidate exists, I will only include a high and low tolerance line.

If no difference in yield exists with which to confidently identify a high and low tolerance line, it is likely because the herbivory imposed during Objective 1 was too mild, as differences in tolerance between some of the examined tomato lines have been observed in earlier studies (Welter and Stegall 1993). In this case, I would opt to select a domesticated line as the low tolerance line and *S. lycopersicum* var. 'cerasiforme' as a high tolerance line, since this trend in tolerance was observed by Welter and Stegall (1993). During the greenhouse experiment, plants will be defoliated either earlier

in development and/or to a more severe extent (>50%) than was imposed in the field, in hopes of triggering a difference in tolerance.

These selected lines will be reared in a greenhouse environment, organized in 20 blocks of eight treatments (+/-microbiome (n=2) X tomato line (n=2) X +/-infestation (n=2)). All plants will be grown in 6" pots containing a 50% field soil, 50% potting soil mixture. Field soil will be collected from the Meigs Horticultural Farm at Throckmorton Purdue Agricultural Center in Lafayette, IN, USA. All potting soil will be steam sterilized prior to seeding. For no microbiome treatments, Meigs soil will be sterilized as well.

Plants will be reared in the greenhouse until budding, upon which infestation treatments will be implemented to impose 50% defoliation by netted tobacco hornworms. Hornworms will again be ordered from Great Lakes Hornworm and reared on artificial diet for one week prior to infestation. I may also use this opportunity to weigh hornworms before and after infestation to obtain proxies for plant resistance. I may also choose to involve a mechanical damage treatment to supplement our herbivory treatments.

Once again, yield will be used as a proxy for tolerance, and I will count and weigh collected ripe fruit on a weekly basis. I may also choose to supplement our use of yield as a proxy for tolerance with biochemical assays that reflect the expression of certain tolerance traits, such as nutrient reallocation upon herbivory. There should be many options for assay targets, but N-transporting amino acids are one option, also used by Steinbrenner et al. (2011). Adding this measure of tolerance traits would both strengthen our identification of tolerant plants and color our understanding of the mechanisms behind expressed tolerance in this system.

Rhizosphere sampling will follow the protocol established in Objective 1. Two, rather than three, samples will be collected, since rhizosphere communities will not be sequenced, and samples will only be used for estimates of microbial biomass.

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