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Mycorrhizal composition influences plant anatomical defense and impacts herbivore growth and survival in a life-stage dependent manner

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ABSTRACT

While arbuscular mycorrhizal (AM) fungi may have a prominent role in trophic ecology, mycorrhizal improvement or reduction on herbivore growth and survival may also be dependent on herbivore's stage of development. *Solanum lycopersicon* (tomato) was grown on sterile background soil treated with either mycorrhizal inoculant (AM+) or non-mycorrhizal control (AM−). Mycorrhizal treatments included four single species of AM-fungi (*Entrophospora infrequens, Funneliformis mosseae, Claroideoglomus claroideum,* and *Racocetra fulgida*) and a mixture of all four species (fungal community). To determine if mycorrhizal treatment indirectly alters the ability of beetle larvae (*Leptinotarsa decemlineata)* to access plant resources, plant damage and trichome density were quantified as plants were infested with a single neonate (early-stage) for 96 hours (h). In a second experiment, beetle growth rate was assessed as plants were infested with a single third-instar (late-stage). After 72 h of late-stage beetle infestation, beetle mass was measured. It was found that early-stage beetles inflicted more damage on AM+ tomatoes. Interestingly, this corresponds with fewer trichomes on AM+ tomatoes, as well as higher early-stage beetle survivorship. Specifically, AM taxon, *C. claroideum* increases herbivory and thereby reduces beetle mortality. Among late-stage beetles, *C. claroideum* does not improve beetle growth nor rate of survival. This suggests that AM taxa that are beneficial to early-stage beetles may not necessarily provide an advantage to late-stage beetles. Taken together, these findings highlight potential dependencies of AM-fungal effects on herbivory and herbivore life history, including growth and life-stage specific survival.

1. Introduction

While ecological communities are at risk of restructuring (Brault and Bourget, 1985; Myers et al., 2007; Smith-Ramesh, 2017; Závada et al., 2017), biotic factors influencing community interactions remain vital to a sustainable ecosystem (Hairston et al., 1960; Paine, 1966; Moore et al., 2004; Mouillot et al., 2013). In terrestrial systems, plant quality, or nutritional value, indirectly influence higher trophic classes (Bukovinszky et al., 2008; Gange and West, 1994). At the same time, plants are impacted by soil organisms that inhabit the root-soil interface (Reynolds et al., 2003; Wagg et al., 2014). Root colonizing fungi, termed arbuscular mycorrhizae (AM), play a pivotal role in trophic interactions, as they can enhance nutritional value of specific resources and impact ecosystem function (Wilson et al., 2009; Bardgett and Van Der Putten, 2014). Through phosphorous enrichment, AM-fungi can sway multiple nutrient economies of multiple trophic links (Hoffmann et al., 2011), which may improve herbivore growth and survival (Borowicz, 1997). Alternatively, AM-fungi may increase plant defense allocation, since enriching the carbon-nutrient balance may make defense budgeting less costly (Gange and West, 1994; Bennett et al., 2006). The first line of plant defense includes volatile organic compounds, the plant cell wall and epidermal protuberances known as trichomes (Dalin et al., 2008; Pozo et al., 2002; Huang et al., 2012). Although trichome density can deter herbivores (Sato and Kudoh, 2017), it is possible that the efficacy of these structures may also depend on herbivore age or development stage.

Determining ways in which AM-fungi can influence herbivory at discrete stages of development may provide additional insight into the role of AM-fungi as it pertains to trophic ecology. As a strong trophic link (Paine, 1980), AM-fungi have the indirect ability to influence pollination, plant-dormancy, nutrient cycles, and even the development rate of holometabolous insects (Barber et al., 2013; Wilson et al., 2009; Rock-Blake et al., 2017; Vannette and Hunter, 2013). For example, the indirect ability of AM-fungi to increase caterpillar's growth rate

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(Vannette and Hunter, 2013) has broad implications as it pertains to food web dynamics. Hence, insect retention period in a particular stage-class may result in vulnerability toward predators or parasitoids that specialize on that particular stage-class (Murdoch et al., 1987). Thus, the ability of AM-fungi to indirectly alter the rate of insect development may relax predation and parasitization pressures. Similarly, plant age can affect resistance or tolerance to natural enemies, including herbivores (Kearsley and Whitham, 1989; Kus et al., 2002), due to greater densities of trichomes on young leaf tissue as opposed to mature leaf tissue (Woodman and Fernandes, 1991; Levin, 1973). Meanwhile, the efficacy of these structures may be strengthened or weakened by select AM-fungal communities.

Though AM-fungal associations can result in plant nutritional enhancement (Jakobsen et al., 1992), colonization of plant roots by AM-fungi can yield induced systemic resistance (ISR) via upregulation of plant defenses (Pieterse et al., 2014; Cameron et al., 2013; Campos-Soriano et al., 2012; Jung et al., 2012). This altered plant physiological state can also enhance density independent mortality, also known as hard selection (Wallace, 1975; Wade, 1985). Hence, upregulation of a given plant defense pathway (i.e. salicylic acid), may prove to be less effective toward a herbivore or a pathogen that is normally deterred by an antagonistic plant defense pathway (i.e. jasmonic acid) (Rojo et al., 2003; Pozo and Azcón-Aguilar, 2007; Campos-Soriano et al., 2012; Wang et al., 2015). For example, AM-fungal colonization increases myrid bug (*Hemipteran*) performance and survivorship on tomato (Prieto et al., 2016), but when tomato was challenged with beet army worm (*Lepidopteran*), AM-fungi lessened herbivore performance (Shrivastava et al., 2015). These examples demonstrate the inconsistencies of ISR. With respect to AM-fungi and foliar pathogen interaction, AM-fungal *spp.*, *Rhizophagus irregularis* has been reported to exacerbate disease symptoms in plants challenged with *Botrytis cinera* and *tobacco mosaic virus* (Shaul et al., 1999). While in another study, AM-fungal *spp. Funneliformis mosseae* was found to mitigate disease symptoms of plants challenged with *cucumber mosaic virus* (Elsharkawy et al., 2012).

To date, studies addressing the role of AM-fungi in trophic dynamics support the importance of resource provisioning as a means of influencing plant enemy interactions (Borowicz, 1997; Bennett et al., 2006; Malik et al., 2016). Applied studies have focused on identifying AM-fungal isolates that mediate bioprotection (Sowik et al., 2016; Spagnoletti et al., 2016; Tchabi et al., 2016; Yuan et al., 2016; Sharma and Sharma, 2016; Mustafa et al., 2016). Though these studies have contributed to our understanding of the role of AM-fungi in plant enemy interactions, very few studies have characterized the differences that may exist when mycorrhizal plants interact with herbivores of two stages of development. Understanding these stage-class interactions can provide insight into life-stage specific trophic dynamics.

In this study, we sought to determine whether AM-fungi affect early or late-stage Colorado potato beetle's (*Leptinotarsa decemlineata*) life history. Here, the following questions are posed (1) Does AM-fungi influence survivorship of early-stage Colorado potato beetle (neonate); (2) can AM-fungi alter the growth rate of late-stage Colorado potato beetle, that has yet to reach adulthood (third instar) (3); if so, can these outcomes be explained by the indirect effect of AM-fungi on trichomes? It is hypothesized that the indirect effect of AM-fungi on trichomes and phytochemical balance will impact lesser-sized neonates. Meanwhile, improvement of plant quality through AM-fungal nutrient provisioning will indirectly increase the growth rate of larger third instars.

2. Methods

2.1. Study system

Solanum lycopersicon (tomato) was the host plant used in this study, primarily because of susceptibility to defoliation by Colorado potato beetle, *Leptinotarsa decemlineata,* (*Coleoptera: Chrysomelidae*). These beetles are native to Mexico, but have expanded their range throughout the United States and into Europe and Asia (Alyokhin, 2009). As herbivores, these beetles are oligophagous and specialize on family *Solanaceae*. Colorado potato beetle damage is most severe following adult emergence and maternal ovipositioning (Hare, 1980). This makes *Solanaceae* plants most vulnerable toward beetles of multiple stage-classes, and warrants the basis of our stage-class trophic interaction study.

The four species of AM-fungi chosen in this study (*E. infrequens, F. mosseae, C. claroideum*, and *R. fulgida*) were previously isolated from Kankakee Sands prairie reserve in Indiana, U.S.A. Species identity was previously confirmed morphologically, and with next generation sequencing technology. In addition, these isolates were worthy candidates because of comparable levels of colonization (Vogelsang et al., 2006) and they are representative of the phylogenetic and genetic diversity of AM-fungi (House et al., 2016; Krüger et al., 2012).

Prior to the experiment, fungal cultures were prepared and bulked on sorghum roots for a full growing season under glasshouse conditions. Sorghum was chosen because it has been previously observed by our research group to promote high spore yield. Mycorrhizal cultures were harvested following senescence of sorghum, which enhanced mycorrhizal sporulation. Aboveground sorghum tissue was then removed, while the belowground soil and mycorrhizal root mix was stored at 4 °C prior to use. Similar to Malik et al. (2016), 50 cm^3 of fungal spores and sorghum root mix were placed between 450 cm^3 of heat sterile background soil (Pennsylvania clay-loam: sand (1:1)). Sand was added to improve drainage in pots and reduce nutrient levels, while increasing plant functional response to AM-fungi. Pot dimensions were 10.8 cm^2 by 10.8 cm in height. Fungal community treatment included an even mixture of all four-fungal species. The control treatment was inoculated with bacteria present in the mycorrhizal inoculum. Similar to Borowicz (1997), bacteria were isolated by washing AM-fungal inoculum through a 38 μm sieve. This fluid was subsequently filtered through Whatman glass fibers (0.7 μm) to remove any remnant fungal structures that may have passage through the 38 μm sieve in the previous step. Thus, the filtered wash ensured that the control (AM−) was positive for bacteria but negative for fungi. AM – microbial wash was then applied to heat sterile control soils. After harvest, presence or absence of mycorrhizae was confirmed with trypan blue staining. This was done for a subset of blocks to confirm colonization (AM+) or non-colonization (AM−).

2.2. Primary consumer: Colorado potato beetle

Colorado potato beetles were isolated from potato fields of Pennsylvania (Chung et al., 2013b). Similar to Chung and Felton (2011) beetles were maintained on tomatoes conditioned in glass house and segregated by stages of development. Since emergence of neonates is related to ambient temperature (Schalk and Stoner, 1979), beetles were manipulated to oviposit eggs under growth chamber conditions (16 h light/8 h dark photo-cycle at 25 °C). Prior to experimental infestation, newly laid eggs were collected from the underside of tomato leaflets and incubated in a petri dish within a growth chamber. Newly hatched neonates were then infested on plants soon after their emergence.

2.3. Microcosm: AM-fungi, plant and early-stage beetle interaction

Two experiments were performed simultaneously in a glass house in a random block design (Table 1). In the first experiment, six-week-old tomato (Arabason organic F1, Harris Seeds) were grown on six mycorrhizal treatments (10 replicates). Plants were first germinated on heat sterile potting soil (Metromix). After two weeks, seedlings were transplanted to experimental or control microcosms where they were assigned treatment groups and exposed to AM+ or AM− for four weeks. After a total of six weeks, single newly hatched beetles (neonate) were placed on tomato where they foraged for 96 h.