Intraspecific competition reduces mycorrhizae conferred susceptibility to herbivores

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Abstract

Mycorrhizal fungi alter their host plant's resistance to herbivores and their competitive ability. However, most studies on how mycorrhizae alter resistance have been conducted in single plant studies, and so the interacting effects of mycorrhizae and competition on constitutive and induced plant resistance is largely unexplored. We tested whether mycorrhizal colonization with Rhizophagus intraradice would alter herbivore performance and the expression of chemical resistance traits in tomato plants with and without intraspecific competition. We treated the plants with jasmonic acid to measure their induced chemical resistance traits which we evaluated by measuring leaf consumption by Trichoplusia ni caterpillars and two traits that affect herbivore performance: protease inhibitors, an antinutritive protein, and carbon/nitrogen ratio, a metric of plant nutritional quality. Mycorrhizae decreased resistance (increased leaf consumption) to herbivores when the plants were not in competition but had no effect in competition. While mycorrhizae reduced protease inhibitors, independent of competition or treatment with jasmonic acid, this did not increase caterpillar feeding. However, mycorrhizae, competition and induction with jasmonic acid interacted to decrease plant nutrition, measured as C/N ratio, which was correlated with caterpillar feeding. Here, we show that mycorrhizae induced decreases in plant nutritional quality; a novel mechanism by which mycorrhizae affect resistance to herbivores. Mycorrhizae and competition interact to decrease plant nutritional quality and alter resistance to herbivores.

Introduction

Arbuscular mycorrhizal fungi (AMF), shape ecosystem community assembly and function by altering plant survival and growth as well as their resistance to antagonists, Arbuscular mycorrhizae are fungal endosymbionts that provide plants with micro and macro nutrients and water in exchange for photosynthate. This can increase plant nutritional quality, growth, and fitness, as well as alter investment in plant defense, thus altering the outcome of their host plant's competitive interactions (Allen and Allen 1984; Crowell et al. 1988; Shi et al. 2016) and their ability to defend themselves against herbivores (Mohr et al. 1998; Vannette and Hunter 2009; Gan et al. 2017).

Arbuscular mycorrhizal fungi have a wide range of effects on the defensive abilities of their plant partners. There is ample evidence in a wide range of systems of both arbuscular mycorrhizae-conferred resistance with mycorrhizae changing traits that affect herbivore performance or preference (Gange and Nice 1997; Gange 2001; Wooley and Paine 2007), and mycorrhizae-conferred susceptibility to herbivores and diseases (Gange et al. 1999, 2005; Babikova et al. 2013). Mycorrhizae can affect not only the constitutive defenses, those that exist regardless of herbivory (Hause et al. 2002; Bennett et al. 2009; Fontana et al. 2009) but also the induced defenses, the production of defensive compounds in response to herbivory (Mohr et al. 1998; Riedel, Groten, and Baldwin 2008). The two main mechanisms by which AMF affect plant resistance to herbivores are by 1) altering expression of hormonally regulated secondary metabolites, and 2) altering plant nutritional composition. Mycorrhizae change their host plant's levels of resistance related hormones (Hause et al. 2002, 2007; Khaosaad et al. 2007) as well as defensive compounds such as β-1,3-glucanase

and phenylalanine (Mohr et al. 1998), aucubin and catalpol (Bennett et al. 2009), and polyphenol oxidase (Minton et al. 2016). Mycorrhizae can alter plant nutritional quality(Gange and Nice 1997) and palatability to herbivores (Gange and West 1994a). While plants can reduce nutrient levels in specific tissue in response to an herbivore (Newingham et al. 2007; Gómez et al. 2010), it is not known whether this response is affected by mycorrhizae.

The few studies that have tested how arbuscular mycorrhizae-induced changes in both nutrients and chemical resistance traits find conflicting results. For example, Wurst et al. (2004) found that arbuscular mycorrhizae in plantago increased phosphorus and carbon in leaves as well as nitrogen in the roots but did not affect resistance compounds. This increase in foliar phosphorus caused accelerated development in *Myzus persicae* aphids. Gange and West (1994a) also found that arbuscular mycorrhizal fungi increased leaf C/N ratios and carbon and nitrogen based chemical defenses in their plant hosts, and subsequently reduced herbivory by the chewing lepidopteran *Arctia caja* . As most herbivores are nitrogen limited, a plant's total nitrogen and C/N ratio can be an important determinants of herbivore preference and performance.

However, the fact that most plants grow in competitive environments further complicates the relationships between plants and herbivores and plants and mycorrhizae. The effects of competition on defense are traditionally viewed as a corollary of the growth defense trade-off hypothesis (Stamp 2003). Limited resources can either be allocated to growth or defense depending on the perceived levels of competition and herbivory. For instance, the ratio of red to far red light, a cue that a plant is being overshadowed by a competitor, leads to decreased sensitivity to jasmonate and thus a reduction of induced defenses and increased levels of herbivory (Moreno et al. 2009). Using predictions from the growth defense trade-off, one might assume that arbuscular mycorrhizae which bring nutrients to their hosts could create a 'high nutrient environment' and thus lead to lower defense levels. However, this is complicated by the fact that mycorrhizae are highly generalist, with single individuals often forming associations with multiple plant individuals and species in a common mycorrhizae network (CMN) (Smith and Read 2008). This often creates a complex and asymmetrical trading network with mycorrhizae distributing resources unequally among its connected hosts and can shift the outcome of plant-plant competitive interactions. Mycorrhizae also shifts the outcome of interspecific competition and thus community assembly (Watkinson and Freckleton 1997 and citations there in; Marler et al. 1999a; Danieli-Silva et al. 2010; Daisog et al. 2012), with mycorrhizal plants gaining a large competitive advantage over less mycorrhizal species (Hartnett et al. 1993). However, mycorrhizae have also been shown to reduce competition and competitive dominance to increase evenness and diversity (Wagg et al. 2011; Stanescu and Maherali 2017). In intraspecific competitive environments, mycorrhizae can preferentially allocate resources to the larger or older plants, (Moora and Zobel 1996, 1998; Weremijewicz and Janos 2013; Weremijewicz et al. 2016).

Previous work has highlighted the conditional nature of the interactions between plants and mycorrhizae. Competition is likely to alter the outcome of plant-mycorrhizae interactions by increasing stress. While competition is nearly ubiquitous in natural and agricultural systems, its role in mycorrhizae conferred resistance to herbivores has not yet been investigated. To address this gap, we employed tomato as a model plant system to investigate: 1) Do mycorrhizae confer resistance to herbivores in competitive environments? and 2) Are the effects of mycorrhizae and competition on plant resistance to herbivores due to constitutive or induced changes in nutritional quality or defensive secondary metabolites?

Materials and Methods

Study system

We conducted this experiment on tomato plants var. Castlemart (*Solanum lycopersicum*). Tomatoes are a valuable field and greenhouse crop which associate with mycorrhizal fungi and have a range of chemical defenses against herbivores. Protease inhibitors are common defenses in tomato leaf tissue (Broadway 1986), which are induced through the jasmonic acid pathway. Since herbivores are commonly nitrogen limited, the inability to digest proteins can significantly retard growth and result in starvation and death.

The efficacy of this defense strategy has been shown in Castlemart tomatoes (Felton et al. 1989, Farmer

and Ryan 1990, Rodriguez-Saona et al. 2010, Shrivastava et al. 2015). We chose to grow the tomatoes in intraspecific competition, as tomato plants grown in agricultural systems will most likely be planted with conspecifics.

Rhizophagus intraradices is a generalist arbuscular mycorrhizal fungus in the sub-phylum Glomeromycotina. It is commercially available and used in organic agricultural systems to increase nutrient uptake and decrease fertilizer use. It has been shown to colonize tomato plants (Caron et al. 1986, Fierro-Coronado et al. 2013, Shrivastava et al. 2015). The mycorrhizal inoculum containing *R. intraradices* spores, and non-mycorrhizal inoculum for controls were obtained from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) at West Virginia University. Both the mycorrhizal inoculum and non-mycorrhizal control were produced in leek trap pots in sand-turface media. The inoculum itself was comprised of sand, turface, leek root fragments, and in the treatment: mycorrhizal spores and hyphal fragments. While the control media did not contain mycorrhizal propagules neither inoculum was sterile.

We used first instar *Trichoplusia ni* caterpillars in a bioassay to measure leaf quality. These larvae were obtained from a colony maintained on artificial diet at Cornell University for many years. *Trichoplusia ni* are generalist Noctuid caterpillars that feed on a wide variety of crop plants including Solanaceous and cruciferous vegetables.

Experimental design

We ran a 2x2x2 fully factorial randomized design with plants grown singly or in competition, with or without mycorrhizae, and with or without induction of jasmonate defenses (n=~24 per treatment, n=191 total).

Castlemart tomato seeds were surface sterilized using a solution of 30% of household bleach in distilled water for 30 minutes. They were then rinsed for 1 minute under running water. We sterilized 2.5x3 cm peat pots, 10 cm plastic pots, and 1:1 sand and turface media (by volume) in an autoclave at 121 at 1 bar of pressure for 90 minutes. This was repeated 3 times with a minimum of 24 hours between each autoclaving. Due to uncertain germination rates, we planted 2-4 tomato seeds into each peat pots filled with either sand-turface mixture and mycorrhizal inoculum or control inoculum in a **3:1** ratio. Following germination, we established our competition and no competition treatments by thinning all treatments so that each pot had either one or two seedlings.

After the tomato plants had germinated and had been thinned, we transferred the peat pots into 10 cm pots that were filled with a 1:1 mixture of sand and turface. Each individual pot was placed in a petri dish to prevent mycorrhizal contamination between plants. We randomized the position of the plants on the greenhouse bench. Plants were watered as needed using tap water and fertilized with 21-5-20 NPK fertilizer diluted to 6ppm every 10-15 days. When the tomato plants were 50 days old, half of them were sprayed with approximately 0.3 g of a 0.5 mM jasmonic acid solution dissolved in a 4% ethanol solution to simulate insect herbivory and induce defenses. Control plants were sprayed with 0.3 g of a 4% ethanol solution. Three days after the application of jasmonic acid, we measured plant height as a metric of plant vigor and harvested leaf tissue for bio and chemical assays. Biomass was not collected as most plant tissue was harvested for assays.

To determine the effect of competition and mycorrhizae on resistance to herbivores, we harvested the terminal leaflet from the second fully expanded leaf using a clean razor blade and placed it in petri dishes on moist filter paper for a bioassay. First-instar cabbage looper caterpillars (*Trichoplusia ni*) were placed on the leaves and allowed to feed. After 3 days, caterpillars were weighed to determine changes in herbivore mass in each of the eight treatments (Thaler and Bostock 2004). We also measured plant damage by measuring leaf area consumed using the grid method (Coley 1983).

To test the potential mechanisms of altered resistance we measured the constitutive and induced resistance traits: Protease inhibitors and C/N ratio. Protease inhibitors are a class of chemical defenses that reduce the digestibility of leaf tissue by breaking down the herbivore's digestive enzymes. Protease inhibitors are produced through the jasmonic acid pathway and can be used to measure expression of this pathway. The C/N ratio on the other hand provides information on both the health of a plant, with a low ratio correlated

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to healthier, plants as well as its attractiveness to herbivores. Most herbivores are nitrogen limited (White 1984), so plant tissue with a high C/N ratio can be less attractive and nutritious (Behmer 2009). Additionally, plants can lower nitrogen levels in tissue in response to herbivory to deter herbivores and protect valuable resources (Newingham et al. 2007; Gomez et al. 2010).

We took the terminal leaflet from the first fully expanded leaf from each plant for analysis using colorimetric protease inhibitor assays (Orians et al. 2000). The remaining leaf tissue was dried and homogenized using 2.3mm zircon beads (RPI) in a ball grinder. Then, 0.8 mg of ground leaf tissue was balled into 4x6mm tin capsules and analyzed using an Elementar analyzer (CHNS) to determine carbon and nitrogen levels.

The soil was dried prior to harvesting roots from each plant to measure levels of mycorrhizal colonization. Roots were stored in ethanol until they could be stained following the ink and vinegar method (Vierheilig et al. 1998). Following staining, samples were stored in a 50% glycerol, 45% water, 5% 1.65 M HCl solution until mycorrhizal colonization was confirmed using microscopy.

Statistics

For plants grown in competition, we averaged the data for both plants before analysis. Caterpillar weight was Ln+1 transformed to fit a normal distribution. Protease inhibitor data were square root transformed to fit a normal distribution and analyzed using a linear model. We ran a series of linear testing the interacting effects of competition, mycorrhizae and induction on each of the response variables: height, protease inhibitor activity, C/N ratio, caterpillar mass, and leaf area consumed. Each of these models were analyzed using a linear model in R using the nlme package (Pinheiro et al. 2018). We also analyzed the effects of the potential mechanisms: protease inhibitor activity and C/N ratio on caterpillar weight and leaf area consumed. For each of these analyses, we ran a full factorial model and dropped non-significant terms in a backwards stepwise fashion. Significant and non-significant results are shown in Table 1. To measure the effect of mycorrhizae on size dimorphism, the data were subset to select only plants grown in competition and we took the difference between plant A and plant B. The absolute value of the difference was log transformed to meet assumptions of normality and analyzed using a linear model with mycorrhizae as a predictor variable. Data were analyzed using R version 3.3.3.

Results

The effect of mycorrhizae and competition on plant damage and herbivore performance

There was an interaction between competition and mycorrhizae such that cabbage looper caterpillars consumed more plant tissue from mycorrhizal associated plants than non-mycorrhizal plants, but only when the plant was not experiencing competition. When the plants were grown in competition, mycorrhizae had no effect on herbivore consumption (Fig. 1, $F_{1,31}=6.28$, p=0.018). Cabbage looper caterpillars on mycorrhizal plants were marginally heavier than those reared on non- mycorrhizal plants ($F_{1,46}=2.9620$, p=0.091), but there was no effect of competition on caterpillar mass ($F_{1,46}<0.001$, p=0.996). While induction increased defensive protease inhibitors ($F_{1,148}=9.55$, p=0.002), it had no effect on amount of leaf tissue caterpillars consumed ($F_{1,42}=0.262$, p=0.611), or their mass ($F_{1,42}=0.324$, p=0.572).
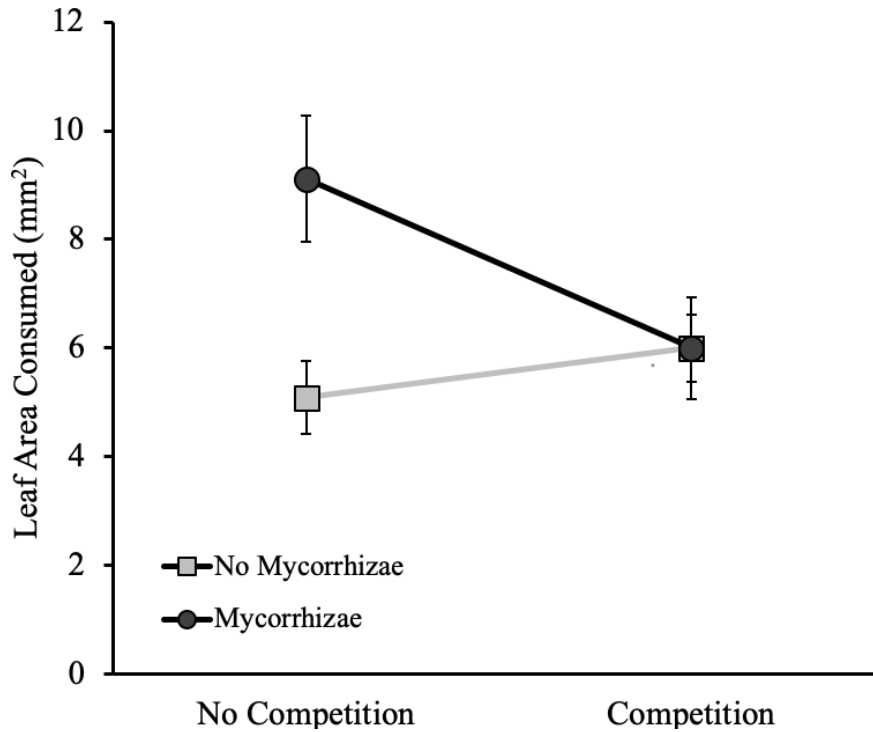
Fig. 1 When competition was not present, cabbage looper caterpillars consumed almost twice as much of leaf tissue from mycorrhizal plants compared to non- mycorrhizal plants. When plants experienced competition, mycorrhizae did not influence leaf area consumed. Symbols represent mean +/- SE.

Mechanisms by which mycorrhizae and competition altered herbivory

Overall, mycorrhizae decreased plant nutritional quality by 19% (Fig 2, $F_{1,78}$=6.40 p < 0.001). Plants inoculated with mycorrhizae and experiencing competition had 13% lower nutritional quality (elevated C/N ratios) in response to jasmonic acid induction (three- way interaction between competition, mycorrhizae and jasmonic acid induction (Fig. 2, $F_{1,78}$=5.35, p=0.024). In contrast, plants grown without mycorrhizae did not respond to JA induction by altering nutritional quality. The % carbon in the leaf tissue remained constant across all treatments (Mycorrhizae $F_{1,45}$=0.229 p=0.634, Competition $F_{1,45}$=1.063 p=0.307, Induction $F_{1,45}$= 0.017 p=0.898) so the changes in the C/N Ratio was driven by changes to the percent Nitrogen (df=53, R^2=0.930, p>0.001).
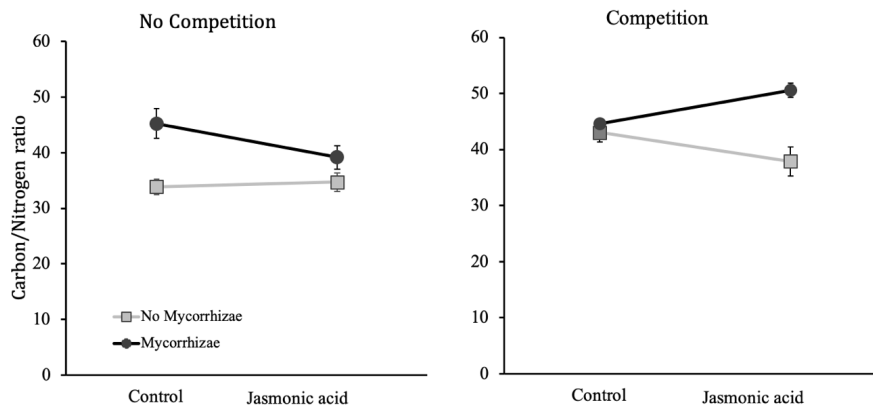

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Fig. 2 Tomato plants grown in competition (A) and tomato plants grown with mycorrhizae had higher C/N ratios compared to tomato plants grown without competition (B), or without mycorrhizae. Tomato plants grown with competition and mycorrhizae induced an increase in carbon nitrogen ratio and thus a decrease in nutritional quality for herbivores. A higher C/N ratio means a lower plant nutritional quality. Symbols represent mean +/- SE.

Overall, plants treated with jasmonic acid had almost double the level of protease inhibitor activity (Fig. 3, $F_{1,148}$=9.55, p=0.002), confirming that the treatment was effective at inducing the plants. Mycorrhizae decreased protease inhibitor levels by 30% regardless of whether the plant was treated with jasmonic acid (Fig. 3, $F_{1,148}$=4.35, p=0.039). Neither competition ($F_{1,148}$=0.057, p=0.451) nor interaction between competition and mycorrhizae ($F_{1,148}$=0.986, p=0. 0.323) impacted protease inhibitors.
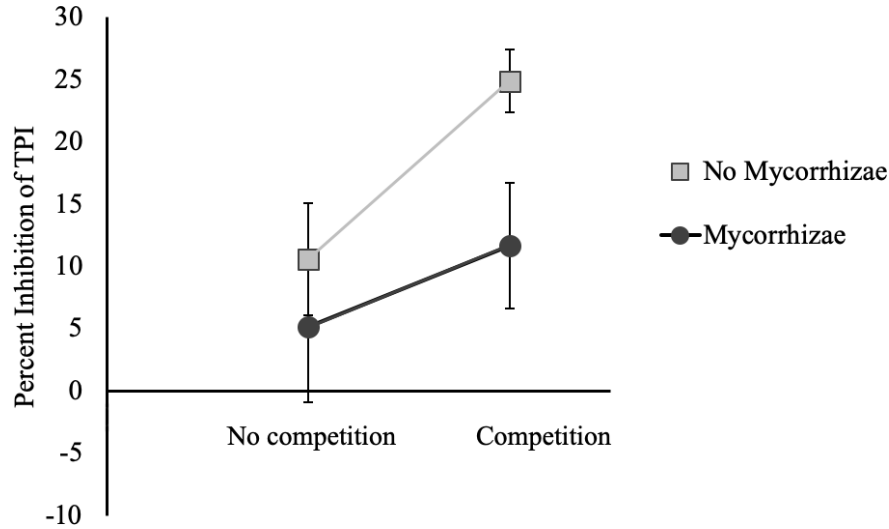

Fig. 3 Percent inhibition is a measure of trypsin protease inhibitor activity, with high inhibition indicating high levels of protease inhibitor activity. Tomato plants grown without mycorrhizae induced protease inhibitors strongly, while plants grown with mycorrhizae did not show significant induction. Symbols represent mean +/- SE.

Since mycorrhizae and competition interacted to affect leaf consumption and affected both plant nutritional content and resistance traits, we tested which traits quantitatively correlated with leaf damage. Cabbage loopers consumed less leaf tissue when C/N ratios were high ($F_{1,31}$=6.99, p=0.013). While we saw a similar effect with nitrogen alone ($F_{1,35}$=5.50, p=0.025), the C/N ratio explained a higher proportion of the variation (R^2=0.16). While treatments with high protease inhibitors also had low cabbage looper leaf consumption, neither constitutive or induced protease inhibitor levels correlated with either cabbage looper feeding or mass gain ($F_{1,29}$=0.781, p=0.383, $F_{1,34}$=0.365, p=0.550).

The effect of mycorrhizae and competition on plant growth

Plants grown with a conspecific were 25% shorter than those grown individually (Fig. 4, $F_{1,174}$=105, p<0.001), confirming that the plants were competing with each other. Mycorrhizae did not alter height (Fig. 4, $F_{1,174}$=1.54, p=0.216). We did not find that mycorrhizae promoted size dimorphism when the plants were grown in competition ($F_{1,100}$=2.30, p= 0.133).
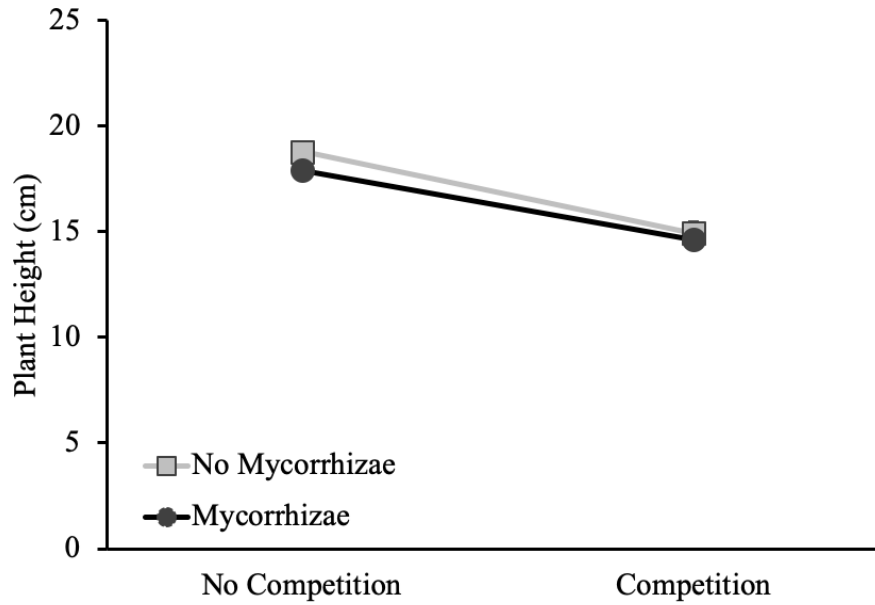
Fig. 4 Tomato plants grown in competition were smaller than those grown singly, while the presence or absence of *R. intraradices* mycorrhizae did not affect height. Symbols represent mean +/- SE.

Table 1: The effect of mycorrhizae, competition, and the jasmonic acid induction treatment on plant defenses (protease inhibitors), nutritional quality (C/N ratio), herbivory (leaf area eaten) and height. Caterpillar mass and Protease inhibitor data were Ln+1 transformed, and square root transformed, respectively, to fit assumptions of normality.

Response variable	Predictor Variable	df	F	p
Leaf area eaten	Mycorrhizae	1,31	2.51	0.123
	Competition	1,31	0.816	0.373
	Jasmonic acid induction	1,31	0.7386	0.3967
	Mycorrhizae x Competition	1,31	6.2835	**0.0176**
	C/N Ratio	1,31	6.9904	**0.0127**
C/N Ratio	Mycorrhizae	1,78	6.4023	**0.0001**
	Competition	1,78	**7.3427**	**0.0084**
	Jasmonic acid induction	1,78	0.4229	0.5175
	Mycorrhizae x competition x jasmonic acid induction	1,78	5.3446	**0.0235**
Protease Inhibitor Activity	Mycorrhizae	1,148	4.3542	**0.0386**
	Competition	1,148	0.5717	0.4508
	Jasmonic acid induction	1,148	9.9495	**0.0024**
	Mycorrhizae x competition	1,148	0.9855	0.3225
	Mycorrhizae x Jasmonic acid induction	1,148	0.3405	0.56041
Plant height	Mycorrhizae	1,174	1.5413	0.2161
	Competition	1,174	105.7047	<0.0001

Discussion

Our results demonstrate that an intraspecific competitive environment alters the effect of the ubiquitous plant-fungi mutualism on plant-herbivore interactions. Arbuscular mycorrhizae decreased plant resistance to herbivores when the plants were grown without competition, but not when they were grown in competi-

tion. We found that arbuscular mycorrhizae decreased plant nutritional quality when grown in competition and induced with jasmonic and this correlated with increased resistance to herbivores. While most studies on mycorrhizae find positive or neutral effects on plant quality, mycorrhizae conferred benefits are highly conditional. Our results are in agreement with the work of Gange and West (1998) and Wurst et al. (2004) who found that, under certain conditions, mycorrhizae can reduce plant resistance to herbivores. Furthermore, we found that mycorrhizae suppress induction of jasmonic acid pathway based defensive protease inhibitors. This supports a number of studies which have found that mycorrhizal do not always benefit plant growth (Ryan and Angus 2003; Bennett and Bever 2007) but can still have effects on plant resistance to herbivores.

Our results support the vast body of literature ((Zimdahl 1980; Weiner 1990; Casper and Jackson 1997; Schwinning and Weiner 1998; Getman-Pickering et al. 2018 among many) showing that competition reduces plant growth. However, our results show no effect of mycorrhizae on size dimorphism in competing plants contradicting the findings by Weremijewicz and Janos (2012), and Ayers, Gange and Aplin who found that mycorrhizae increased size dimorphism in Andropogon gerardii and decreased size dimorphism in *Plantago lanceolate*r espectively. However, both studies do not use entangled root systems, which may account for the difference.

Mycorrhizae suppressed induction of defensive protease inhibitors. These results support the growing body of work that shows that the relationship between arbuscular mycorrhizae and plants can be antagonistic, especially in tri-trophic frameworks. Arbuscular mycorrhizae have been shown to suppress defensive compounds and increase susceptibility of their hosts to a variety of insect herbivores, including garden tiger moths, chrysanthemum leaf-miners and garden buckeyes (Gernns et al. 2001; Gange et al. 2003; Bennett and Bever 2007; Hartley and Gange 2009; Gehring and Bennett 2009). Similar effects have been shown in mycorrhizae-plant-pathogen studies (Volpin et al. 1995; Shaul et al. 1999; Gernns et al. 2001). In our study and others, a suppression of defenses did not necessarily mean a decrease in overall growth. The decrease in induction and the fact that mycorrhizae did not increase growth suggest a more parasitic relationship between the plant and fungus in this experiment.

The fact that arbuscular mycorrhizae did not alter the plant's constitutive defenses but did suppress induced defenses supports previous research that finds that the formation of the association between plants and arbuscular mycorrhizae alters defensive hormone signaling in the plant. As protease inhibitors are known to be regulated by the jasmonate pathway in tomato plants, it is not surprising that protease inhibitor induction was altered by arbuscular mycorrhizae. However, while overall trends in protease inhibitor activity matched the treatment effects on cabbage looper caterpillars, protease inhibitor activity in each individual plant was not correlated with caterpillar weight or the leaf area they consumed. This result implies that while arbuscular mycorrhizae are altering expression of the jasmonic acid pathway, the protease inhibitors are not the primary chemicals responsible for the effect on cabbage loopers.

The presence of mycorrhizae and competition alter the way that plants induce changes in nitrogen levels and subsequently the C/N ratio. While carbon levels independently didn't impact herbivory, high ratios of carbon to nitrogen decreased cabbage looper feeding. As herbivores are frequently nitrogen limited, increased C/N ratios in foliage can be an effective defense strategy for deterring herbivory. Plants associated with mycorrhizae may have lower nutritional levels due to competition between the two organisms for limited nutrients in the soil media (Kaye and Hart 1997). This novel demonstration that mycorrhizae can suppress leaf nutrient content following herbivory provides a new mechanism for the effects of mycorrhizae on plant nutrition and resistance. It is particularly interesting that mycorrhizae can affect constitutive and induced defenses quite differently.

In our study, competition had no effect on induction of protease inhibitors or leaf area consumed. This supports the growing body of literature that fails to find support for the competition-defense tradeoff (Viola et al. 2010). While our study only addressed intraspecific competition, chosen because it is common in agricultural settings, weeds and intercropping systems can result in interspecific competition for the target crop. We predict that in interspecific competitive situations, the effects of arbuscular mycorrhizae on herbivory might not be suppressed for both partners as we found in this study. Rather, the stronger partner

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might continue to see a strong effect of arbuscular mycorrhizae on herbivory, while the weaker partner will not, depending on the strength of the interaction as mycorrhizae often preferentially give nutrients to one partner over another (Marler et al. 1999b).

Our results, along with the many papers cited above, show that tri-trophic effects of arbuscular mycorrhizae are context dependent, and while previous studies have shown that the species of insect and arbuscular mycorrhizae can alter the outcome of interactions, our work shows that the presence of competition can change the direction of the interaction between plant and mutualist. Due to the challenges of working with mycorrhizae, the vast majority of studies on mycorrhizae and herbivory have been conducted in greenhouse experiments where the plants are grown individually (Riedel et al. 2008; Bennett et al. 2009; Tomczak et al. 2016), although a few have studied this phenomenon in field settings (Gehring & Whitham, 1991; Gange & West, 1994; Gange *et al.* , 2005). As plants mostly exist in competition, either with conspecifics or other species, it is important that future tests of the effect of mycorrhizae-conferred resistance to herbivores account for the role of competition.

Author Contribution

ZGP and JT conceived of the project. ZGP and DR designed the methods and carried out the experiment and assays. ZGP ran the statistics and ZGP and JT wrote the manuscript with input from all DR.

Data Management

Data will be archived with Dryad.

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