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Variable Effects of Mycorrhizal Fungi on Predator-Prey Dynamics Under Field Conditions

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Abstract

1. Interactions between herbivores and their predators are shaped, in part, by plant phenotype. Consequently, ubiquitous symbionts of plants belowground, such as arbuscular mycorrhizal fungi (AMF), may influence interactions aboveground between predators and their prey by altering plant phenotype. However, the ecological relevance of belowground organisms on predator-prey interactions under field conditions remains unclear.
2. We assessed how AMF influence herbivore-predator interactions through a field experiment.

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3. We planted two milkweed species (*Asclepias curassavica*, *A. incarnata*) provided with different amounts of AMF inoculum (zero, medium, high) in a randomized block design. We added aphids to plants and reduced predator pressure weekly for five weeks to evaluate effects of AMF on predator recruitment. We then allowed herbivore-predator interactions to reestablish naturally for the remainder of the season to examine whether AMF-mediated variation in predator recruitment influenced the suppression of aphid populations.
4. AMF availability in soils mediated interactions between predaceous aphid midge flies (*Aphidoletes aphidimyza*) and their aphid prey (*Aphis nerii*), but effects were plant species-specific. On *A. curassavica*, by mid-season, midges were recruited most strongly on plants under medium AMF availability and least on plants under high AMF availability. In contrast, each midge killed fewer aphids with increasing aphid density on medium AMF plants, but killed more aphids with increasing aphid density on high AMF plants. In combination, aphid mortality rates imposed by midges were greatest on medium AMF plants, followed by high and zero AMF plants. By comparison, on *A. incarnata*, the recruitment of midges was strongest on high AMF plants and weakest on medium AMF plants. AMF had no effect on the number of aphids killed per midge, relative to aphid density, so mortality rates of aphids imposed by midges mirrored recruitment. Rates of decline in aphid populations following predator recolonization were associated with midge densities, as well as lacewing and syrphid densities, which were unaffected by AMF availability. Therefore, effects of AMF on aphid population decline were not a simple function of AMF-midge interactions.
5. Our findings demonstrate that the availability of AMF in soils has pervasive, but complex, effects on predator-herbivore dynamics in the field.

Keywords: arbuscular mycorrhizal fungi (AMF), microbe-plant-insect interactions, predation, numerical response, functional response, *Aphis nerii, Aphidoletes aphidimyza, Asclepias*

Introduction

Multitrophic species interactions are shaped by a combination of bottom-up forces, such as resource availability, and top-down forces, such as predation and parasitism (Hunter & Price,

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1992). In terrestrial systems, organisms below ground influence the strength of these forces on communities above ground by altering plant phenotype (Grunseich, Thompson, Aguirre, & Helms, 2020; Heinen, Biere, Harvey, & Bezemer, 2018). In doing so, soil microbes influence the performance of insect herbivores above ground, and alter the attraction of predators and parasitoids to their herbivore prey (Koricheva, Gange, & Jones, 2009; Rasmann, Bennett, Biere, Karley, & Guerrieri, 2017). Similarly, insect herbivores, and their interactions with natural enemies aboveground, alter the abundance and composition of microbial communities in soil and roots (Hoffmann, Vierheilig, Peneder, & Schausberger, 2011; Wilkinson, Miranda, Ferrari, Hartley, & Hodge, 2019). However, we lack a general understanding of the ecological relevance of belowground organisms on herbivore-natural enemy interactions in the field, despite the apparent ubiquity of such interactions (Heinen et al., 2018).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous symbionts of most plants below ground, associating frequently with over eighty percent of plant species (Smith & Read, 2008). AMF provide nutrients, such as phosphorous, to plants in exchange for sugars (Smith & Read, 2008). In doing so, AMF interact with plant defensive signaling pathways and alter plant nutrient uptake, thereby affecting plant defensive and nutritional traits (Meier & Hunter, 2018a; Schweiger, Baier, Persicke, & Müller, 2014). AMF frequently stimulate plant growth and mitigate abiotic and pathogen stress (Smith & Read, 2008), but effects of AMF on plant growth and defense range from beneficial to detrimental, depending on the environment (Hoeksema et al., 2010), the identity of plants and AMF (Anacker, Klironomos, Maherali, Reinhart, & Strauss, 2014), and the density of AMF inoculum available to plants (Vannette & Hunter, 2011, 2013).

AMF invoke variable effects on herbivore performance. For instance, AMF often reduce the performance of chewers, but improve the performance of phloem feeders (Koricheva et al., 2009). However, most studies to date have considered only how AMF affect herbivores in the absence of natural enemies, although herbivore population dynamics are often driven by their natural enemies (Turchin et al., 2003). By altering volatile emissions from plants, AMF can increase the attraction of natural enemies to plants (Babikova et al., 2013; Hoffmann, Vierheilig, & Schausberger, 2011a; Prieto et al., 2017; Schausberger, Peneder, Jürschik, & Hoffmann, 2012) even in the absence of herbivores (Guerrieri, Lingua, Digilio, Massa, & Berta, 2004). In contrast,

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by increasing plant size and quality, AMF can reduce the searching efficiency of natural enemies (Gange, Brown, & Aplin, 2003) and reduce rates of parasitism by improving herbivore performance (Moon, Barnouti, & Younginger, 2013). Furthermore, AMF often improve the performance of natural enemies, enhancing the population growth of predators (Hoffmann, Vierheilig, & Schausberger, 2011b) and the successful development of parasitoids (Bennett, Millar, Gedrovics, & Karley, 2016; Hempel et al., 2009). Indeed, in some cases, AMF-mediated increases in herbivore performance may be compensated for by a combination of enhanced predator populations and higher plant tolerance to damage, ultimately leading AMF to benefit their host plants (Hoffmann, Vierheilig, Peneder, et al., 2011). However, in other cases, AMF-mediated increases in plant defenses can enhance the sequestration of defensive metabolites by herbivores, which may increase herbivore resistance to their natural enemies (Meier & Hunter, 2018b). Therefore, to understand the potential impacts of AMF on aboveground communities, we must consider the effects of AMF on multitrophic interactions under field conditions.

Here, we evaluate how AMF influence herbivore-predator interactions in the field on milkweed (*Asclepias*) species. Milkweeds provide an ideal system in which to assess effects of AMF on multitrophic interactions. Milkweeds produce cardenolides, bitter tasting steroids that disrupt the functioning of sodium-potassium channels in animal cells (Agrawal, Petschenka, Bingham, Weber, & Rasmann, 2012). Milkweeds show strong, AMF-mediated variation in both constitutive and induced cardenolide concentrations (Meier & Hunter, 2018a; Tao, Ahmad, de Roode, & Hunter, 2016; Vannette & Hunter, 2013) and volatile emissions (Meier & Hunter, 2019). Milkweeds are attacked by a community of specialized herbivores, such as oleander aphids (*Aphis nerii*), which can tolerate and sequester cardenolides, but are still negatively affected by cardenolides at high concentrations (Agrawal, 2004). In the field, *A. nerii* are killed by a suite of generalist predators and parasitoids, including lacewings (Neuroptera), syrphids (Diptera), coccinellids (Coleoptera), spiders (Araneae), aphid midge flies (*Aphidoletes aphidimyza*, Diptera), and parasitoid wasps (Hymenoptera) (Malcolm, 1992; Mohl, Stenoien, & Heimpel, 2020). Many of these natural enemies are affected negatively by aphid sequestration of high concentrations of cardenolides (Malcolm, 1992; Mohl et al., 2020). Importantly, AMF-mediated increases in foliar cardenolide concentrations reduce the performance of oleander

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aphids, but also enhance their sequestration of cardenolides (Meier & Hunter, 2018b), indicating that AMF may affect interactions between oleander aphids and their predators in the field.

We assessed how AMF influence herbivore-predator interactions through a field experiment. We grew plants of two milkweed species provided with different amounts of AMF inoculum in a randomized block design in the field. We added aphids to plants and reduced predator pressure weekly by removing predators for five weeks to evaluate how AMF affect 1) aphid population growth and 2) predator recruitment over the season. We found previously that AMF influence the per capita growth rates of *A. nerii* nonlinearly on these milkweed species under greenhouse conditions (Meier & Hunter, 2018b). Therefore, we hypothesized that aphid populations, under reduced predator pressure, would be lowest on plants under medium AMF availability and greatest on plants under high AMF availability. In addition, we expected that predator recruitment would be greater on plants inoculated with AMF.

We then allowed herbivore-predator interactions to reestablish naturally for the remainder of the season to examine whether AMF-mediated variation in predator recruitment influences the suppression of aphid populations. Specifically, we asked how AMF influence the 1) functional responses of predators to aphids, 2) rates of aphid mortality imposed by predators, and 3) overall rates of aphid population decline. We did not have specific predictions for the outcomes of these interactions, as AMF effects on plant volatile profiles (Meier & Hunter, 2019), aphid population growth, and aphid sequestration of defensive metabolites (cardenolides) (Meier & Hunter, 2018b) are specific to the milkweed species and level of AMF inoculum availability. The overall influence of AMF on aphid-predator interactions will depend on the relative strength of AMF effects on the bottom-up (i.e. plant quality) and top-down (i.e. predator recruitment and performance) forces acting on aphid populations.

Methods

Plants and Insects

We used two North American milkweed species (*Asclepias curassavica* and *A. incarnata*) that show constitutive and AMF-mediated variation in defenses and nutritive quality (Meier & Hunter, 2018a; Tao et al., 2016). *Asclepias incarnata* seeds were collected from naturally

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occurring populations in Emmet County, MI. *Asclepias curassavica* seeds were purchased from Victory Seeds (Molalla OR, www.victoryseeds.com). We obtained our AMF inoculum from Mycorrhizal Applications (Grants Pass, OR, USA), which is advertised to contain four AMF species including *Rhizophagus intraradices*, *Funneliformis mosseae*, *Glomus aggregatum*, and *Claroideoglomus etunicatum* (33 spores of each AMF species per gram inoculum). However, we later found this mix to contain only *Funneliformis mosseae* (Meier & Hunter, 2018b). Milkweed species grow in habitats that host AMF taxa (Öpik, Moora, Liira, & Zobel, 2006), and can form associations with this cosmopolitan AMF species (Meier & Hunter, 2018a; Tao et al., 2016), although the frequency of such interactions is unknown.

Experimental Protocols

Seeds of *A. incarnata* were cold stratified for 6 weeks at 4°C (seeds of the tropical *A. curassavica* were not cold stratified), surface sterilized in 5% bleach, and germinated at room temperature. In mid-April 2015, we planted individual seedlings into conical deepots (D40H, Steuwe and Sons, Inc., Corvalis, OR, USA) filled with 600 ml of a mix of autoclaved soil (Metro-Mix 360, MetroMix Sun Gro Horticulture Canada CM Ltd., Vancouver, BC, Canada) and sand (5:3) containing AMF inoculum. We homogenized 4.20 g of autoclaved AMF inoculum (zero treatment), 1.20 g live and 3.00 g autoclaved inoculum (medium treatment), or 4.20 g live inoculum (high treatment) in 200 ml of autoclaved soil and sand, which was placed between 400 ml autoclaved soil and sand. We returned the natural bacterial community to the soil by adding 20 ml of a suspension of 100 ml soil in 1 L deionized water filtered through an ultra-fine sieve (32 μm) to remove AMF hyphae and spores. We grew plants in a greenhouse under 15L:9D, watered plants *ad libitum*, and fertilized them weekly with 30 ml of 15-0-15 (N-P-K, 567 ppm) dark weather fertilizer (JR Peters Inc., Allentown, PA).

In mid-July 2015 (3-month old plants), we harvested a subset of plants (10 replicates of each treatment combination, N=60) to measure initial plant traits and AMF colonization (below). Plants were harvested in four temporal blocks, each separated by one day, with treatments equally represented within blocks. Concurrently, we placed 19-23 replicates of each plant species*AMF treatment combination in a mowed field at the Matthaei Botanical Gardens (Ann Arbor, MI). Four to six replicates of each treatment combination were placed in each of four

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spatial blocks. Plants were elevated above the soil surface in 24-inch plant props (Luster Leaf, www.lusterleaf.com) to prevent colonization by natural AMF (Fig. S1). One reproductive oleander aphid (*A. nerii*) was placed on each plant and allowed to reproduce overnight. The following day, reproductive aphids were removed, and colonies were thinned to five aphid offspring per plant. Dead or missing first instar aphids were replaced for the first three days.

For the first five weeks, we counted *Aphis nerii* and predaceous arthropods and their eggs weekly. We assigned predators to general taxonomic groups, including spiders, coccinellids, lacewings, aphid midge flies, and syrphids. As we counted, we removed all predators and their eggs using a paintbrush, without disturbing aphid colonies. Following these five weeks of reduced predator pressure, we continued to count arthropods, but did not remove predators or their eggs for the remainder of the growing season (four weeks). As a metric of aphid predation by aphid midge flies, we also counted aphids killed by midges, which have characteristic black shells that persist on plants for approximately one week. Throughout the experiment, we measured plant height biweekly.

We first assessed the effects of our AMF treatments on plant traits and AMF colonization from each of the milkweeds harvested in July (pre-field exposure). We measured foliar cardenolide concentrations, leaf water content, specific leaf mass, foliar carbon, nitrogen, and phosphorous concentrations, above and belowground biomass, plant height and leaf number, and AMF colonization of the roots for each plant following Meier & Hunter (2018a). At the end of the growing season (October 2015), we harvested the field plants to measure plant biomass, foliar cardenolide concentrations, and the proportion of roots colonized by AMF. We were unable to measure late-season nutrient content of leaves because many plants had begun to senesce by the time of the final harvest. Detailed methods of the analyses of plant traits and AMF colonization are provided in the Supporting Information.

Data Analyses

In the analyses that follow, all generalized linear mixed models were performed in SAS 9.4 (SAS Institute, Cary, NC, USA) using the function GLIMMIX. All general linear mixed models were analyzed in R v 3.6.1 (R Core Team, 2019) using the 'lmer' function in the 'lme4' package

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(Bates, Maechler, Bolker, & Walker, 2015). Significance of treatments was assessed using the 'anova' function in the 'lmerTest' package (Kuznetsova, Brockhoff, & Christensen, 2017). Residuals were checked for normality and homogeneity of variance. Data were natural log-transformed when necessary. Sample sizes for all analyses are reported in the Supporting Information.

Analyses of AMF Colonization and Plant Traits

To evaluate effects of AMF availability and milkweed species on proportion root colonization by AMF and plant traits and early and late in the season, we used linear mixed models, with block as a random effect. Each trait was the dependent variable, and milkweed species, AMF treatment, and their interaction were fixed effects. Cardenolide concentrations were analyzed separately for each plant species due to substantially different expression in each species.

Insect Analyses: Reduced Predator Pressure (Weeks 1-6)

Because many aphid colonies went extinct even during the six weeks of reduced predator pressure, we first analyzed whether aphid colony persistence over these six weeks varied with AMF availability and milkweed species. We used a generalized linear mixed model with a binomial distribution and logit link function. Whether or not an aphid colony persisted was the binary dependent variable, and milkweed species, AMF inoculum availability, and their interaction were fixed effects. Block was designated as a random effect. Next, using only those plants on which aphid colonies persisted, we evaluated effects of AMF availability and milkweed species on aphid population growth under reduced predator pressure over time (Weeks 1-6) using a generalized linear mixed model with a negative binomial distribution, log link function, and autoregressive covariance structure. AMF, milkweed species, week, and their interactions were fixed effects. Block and the identity of individual plants were random effects. To evaluate whether variation in aphid abundance was mediated by plant size, we repeated the above analysis, but added plant height to the model as a covariate.

We assessed variation in predator recruitment over the season by summing together counts of all life stages of aphid midge flies, coccinellids, lacewings, syrphids, and spiders per week. We included predator eggs in these calculations because we would otherwise underestimate total

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predator recruitment; only eggs laid right after predator removal the previous week could hatch by our survey the following week. We evaluated effects of AMF availability and milkweed species on total predator recruitment over time (Weeks 1-6) using the same generalized linear mixed model structure that we used for aphids. To evaluate whether variation in predator recruitment was mediated by aphid abundance, we repeated the above analysis, but added aphid density per week to the model as a covariate.

Aphid midge flies (*Aphidoletes aphidimyza*) were the only predators to occur on most plant individuals over the sampling period. Therefore, we could only assess effects of AMF and plant species on the recruitment of midge flies over time. We evaluated weekly midge fly recruitment relative to aphid density (i.e. numerical response of midges to aphids, *sensu* Holling, 1959) using a generalized linear mixed model with a negative binomial distribution, log link function, and autoregressive covariance structure. AMF, milkweed species, week, aphid density, and their interactions were designated as fixed effects. Block and the identity of individual plants were designated as random effects. Midges were present beginning the second week of the experiment, so analyses included only weeks two through six.

To consider effects of AMF and milkweed species on the recruitment of other taxonomic groups of predators, we summed counts of all life stages of each predator taxonomic group (i.e. coccinellids, lacewings, syrphids, and spiders) over the initial six weeks. For each predator group, we performed separate, generalized linear mixed models with a negative binomial distribution and log link function. The cumulative abundance of each predator group was our dependent variable. AMF availability, milkweed species, cumulative aphid density over the six weeks, and their interactions were fixed effects, and block was a random effect.

Insect Analyses: Full predator pressure reestablished (Weeks 6-9)

We evaluated effects of AMF and milkweed species on the number of aphids killed per midge relative to aphid density (i.e. functional response of midges to aphids, *sensu* Holling, 1959) using a linear mixed model. Aphid density in Week 6, AMF, milkweed species, and their interactions were fixed effects and block was a random effect. We calculated the number of aphids killed per midge by dividing the number of aphids killed by midges in Week 7 (the first week following

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cessation of predator removal) by the number of midges recruited to plants in Week 6. We focused on aphid dynamics between Weeks 6 and 7 because a significant number of aphid colonies were driven extinct within a week of stopping the removal of predators. Plants on which no midges were counted in Week 6 were excluded from the analysis. In addition, we calculated the mortality rate of aphids imposed by midges using a generalized linear mixed model with a negative binomial distribution and log link function. The number of aphids killed by midges in Week 7 was the dependent variable, AMF availability, milkweed species, aphid density the previous week (Week 6), and their interactions were fixed effects, and block was a random effect.

To consider whether AMF influenced the rate of decline of aphid populations in the week following the cessation of predator removal, we calculated the per capita rate of change in aphid populations (r) as ln((Week 7 aphid population + 1) / Week 6 aphid population). We added one to the Week 7 counts because some aphid colonies had been driven to zero. We evaluated whether the per capita rate of decline of aphid populations varied among AMF treatments and milkweed species using a linear mixed model with AMF, milkweed species, and their interaction as fixed effects and block as a random effect. To determine which predators drove this decline, we performed multiple regression with the rate of decline as our dependent variable, the abundance of midges, syrphids, lacewings, and coccinellids in Week 6 as fixed effects, and block as a random effect. We did not include spider abundances in this model because spider and midge abundances were correlated ($r=0.24$, $P=0.035$). Midge, syrphid, lacewing, and coccinellid abundances were not correlated with one another ($r < 0.1$). In addition, we evaluated whether the persistence of aphid colonies to the end of the season (Weeks 7-9) varied with AMF availability or plant species. We used a generalized linear mixed model with a binomial distribution and logit link function. Whether or not an aphid colony persisted was the binary dependent variable, and milkweed species, AMF inoculum availability, and their interaction were fixed effects. Block was designated as a random effect.

Results

AMF Colonization

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Our AMF treatments were successful in generating variation in AMF colonization. At the beginning of the experiment (mid-July, 3-month old plants), plants without AMF were never colonized (0.0% ± 0.0) and roots of plants under medium and high AMF availability were 47.1% (± 6.0) and 60.1% (± 3.7) colonized, respectively (AMF: $F_{2,52}$=56.34, P<0.0001; Tables S1, S2). By the end of the season (October, 5-month old plants), plants without AMF were rarely colonized (0.1 % ± 0.0) and roots of plants under medium and high AMF availability were 57.2% (± 2.4) and 62.7% (±1.7) colonized, respectively ($F_{2,69}$=428.1, P<0.0001; Tables S3, S4). Colonization by AMF never varied between plant species, and colonization with arbuscules followed total colonization closely (Tables S1-S4).

Plant Traits

At the beginning of the experiment, medium and high AMF availability increased foliar phosphorous concentrations in *A. curassavica* plants by 43 and 58%, respectively. In *A. incarnata* plants, high AMF availability increased foliar phosphorous concentrations by 16%, but medium AMF availability did not affect foliar phosphorous concentrations (AMF*Plant Species: $F_{2,50.8}$=4.72, P=0.0132; Tables S1, S2). In addition, inoculation with AMF reduced leaf toughness in both plant species, especially under high AMF availability ($F_{2,48}$=5.64, P=0.0063; Tables S1, S2). Foliar carbon, nitrogen, and cardenolide concentrations, and plant biomass, height, and leaf number were unaffected by AMF, but all traits, except foliar nitrogen concentrations, varied between plant species (Tables S1, S2). At the end of the season, inoculation with AMF did not affect plant biomass or the production of foliar cardenolides, although these traits did vary between plant species (Tables S3, S4).

Reduced Predator Pressure (Weeks 1-6)

Neither the probability that aphid colonies would persist, nor the population growth of the colonies that persisted, were affected by plant species or AMF availability (Tables S5-S7). Total predator recruitment varied over the season differentially between plant species (Plant Species*Weeks $F_{5,15}$=6.13, P=0.0028; Tables S6, S7), but these effects were driven solely by variation in aphid abundance; effects of plant species on predator recruitment were no longer significant once accounting for increased recruitment of predators to plants with more aphids (Plant Species*Weeks $F_{5,15}$=2.72, P=0.0610, Aphids $F_{1,419}$=132.27, P<0.0001).

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The effects of AMF on the numerical responses of midge flies to their aphid prey varied between milkweed species and over time (Plant Species*AMF*Weeks*Aphids: $F_{8,321}$=3.77, P=0.0003; Table 1, Fig. 1). By mid-season (Week 6), midge recruitment increased with aphid density on *A. curassavica* plants under zero and medium AMF availability, but midge recruitment was unaffected by aphid density on plants under high AMF availability. The numerical response of midges to aphids was 1.2 fold higher on *A. curassavica* plants under medium AMF availability than plants without AMF. In contrast, on *A. incarnata*, midge recruitment increased with aphid density on plants under high AMF availability and without AMF, but was unaffected by aphid density on plants under medium AMF availability. The numerical response of midges to aphids was 3.1 fold higher on *A. incarnata* plants under high AMF availability than plants without AMF. Early in the season, the recruitment of midges relative to aphid density was affected minimally by the amount of AMF available to either plant species (Fig. 1).

In contrast to midges, the cumulative recruitment of coccinellids over the season varied with AMF availability similarly between plant species. Coccinellid recruitment decreased with aphid density on plants under medium AMF availability, but did not vary with aphid density on plants under high or zero AMF availability (AMF*Aphids $F_{2,62}$=8.16, P=0.0007; Fig. S2). The cumulative recruitment of lacewings, syrphids, and spiders over the season were unaffected by aphid density, plant species, or AMF availability (Table S8).

Full Predator Pressure (Weeks 6-9)

Once predators were permitted to remain on plants, we observed complex variation in midge fly functional responses to aphid density among plant species and AMF treatments, although effects were statistically marginal (Plant Species*AMF*Aphids: $F_{2,48}$=2.87, P=0.0665; Table 2, Fig. 2). To clarify this complexity, we re-ran the models separately for each milkweed species. We determined that the per capita consumption of aphids by midge flies on *A. incarnata* is independent of AMF and aphid density (AMF*Aphids $F_{2,20}$=1.24, P=0.3114, Table S9). In contrast, we found evidence that the functional responses of midge flies to aphid densities varied with AMF availability on *A. curassavica* (AMF*Aphids $F_{2,27.7}$=3.22, P=0.0551, Table S9). Specifically, midges exhibit positive functional responses to increasing aphid density under high

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AMF availability, increasing their per capita consumption of aphids (Fig. 2). Midges exhibit no such increases in their per capita consumption rates under medium or zero AMF availability (Fig. 2).

The effects of AMF on midge numerical and functional responses were generally reflected in the aphid mortality rates imposed by midges. Aphid mortality rates varied among AMF treatments differentially between milkweed species (Plant species*AMF*Aphids $F_{2,62}$=3.02, P=0.0562, Fig. 3, Table 2). Specifically, aphids on *A. curassavica* plants suffered notably higher mortality rates under medium AMF availability (0.31 aphids killed/aphid), than on plants under high AMF availability (0.16 aphids killed/aphid), or on those without AMF (0.08 aphids killed/aphid). On *A. incarnata*, the impacts of AMF on aphid mortality rates from midges mirrored the impacts of AMF on midge numerical responses; aphids experienced their highest mortality rates on *A. incarnata* plants under high AMF availability (0.23 aphids killed/aphid), followed by plants without AMF (0.19 aphids killed/aphid), and then those under medium AMF availability (0.002 aphids killed/aphid).

Although statistically marginal, there was some evidence that rates of aphid population decline following predator release varied among AMF treatments and plant species (AMF*Plant Species $F_{2,70.1}$=2.84, P=0.0655; Fig. 4, Table S10). After we stopped removing predators, aphid populations on *A. curassavica* plants declined at a 54% slower rate on medium AMF plants when compared to plants under high AMF availability or those without AMF. In contrast, aphid colonies on *A. incarnata* plants declined at a 57% faster rate on plants under medium AMF than on plants under high AMF availability or those without AMF. Interestingly, the patterns of aphid mortality imposed by midges (Fig. 3) do not predict the patterns of aphid population decline (Fig. 4) among AMF treatments and milkweed species. This may reflect the impacts of other predator taxa that, while unaffected by AMF treatment, nonetheless were correlated with rates of aphid decline. Indeed, the decline rate of aphid populations was correlated positively with midge, syrphid, and lacewing abundances (Midges $F_{1,69.7}$=14.54, P= 0.0003; Syrphids $F_{1,69.1}$=3.84, P=0.054; Lacewings $F_{1,68.2}$=3.84, P=0.054). Neither AMF availability nor plant species influenced the probability that aphid colonies would persist to the end of the growing season (Table S11).

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Discussion

We demonstrate that the availability of AMF in soils has nonlinear effects on the interaction between predaceous midge flies (*Aphidoletes aphidimyza*) and their aphid prey (*Aphis nerii*) under field conditions. Such nonlinearities in multitrophic interactions are to be expected, as the association between plants and AMF is inherently nonlinear. The amount of AMF available to plants may affect the ratio of phosphorous benefit to carbon cost for plants, leading to nonlinear responses in plant phenotype, and corresponding herbivore performance, to AMF availability (Vannette & Hunter, 2011, 2013). Notably, the effects of AMF availability on aphid-midge interactions were also milkweed species-specific, suggesting that it may be challenging to generalize the effects of AMF on predator-prey dynamics, even among congeneric plant species.

Overall, effects of AMF availability on aphid midge fly recruitment strengthened over the season, but varied between plant species. On *A. curassavica*, by mid-season, the numerical responses of midges to variation in aphid density were strongest on medium AMF plants, and weakest on high AMF plants (Fig. 1). In notable contrast, the functional responses of midges were weakest on medium AMF plants and strongest on high AMF plants (Fig. 2). In combination, the aphid mortality rates imposed by midges were greatest on medium AMF plants, followed by high and zero AMF plants (Fig. 3). By comparison, on *A. incarnata*, the numerical responses of midges to variation in aphid density were strongest on high AMF plants and weakest on medium AMF plants (Fig. 1). Because AMF availability had no effect on the functional response of midges to aphids on *A. incarnata* (Fig. 2), mortality rates of aphids imposed by midges mirrored recruitment (Fig. 3).

Despite the effects of AMF on midge numerical responses, and their subsequent predation levels on aphids, AMF-midge interactions could not explain the rates of decline in aphid populations when predators were allowed to recolonize plants. This is consistent with our findings that AMF availability did not influence the recruitment of other predator taxa, such as lacewings and syrphids. Declines in aphid populations were associated with densities of lacewings and syrphids, as well as those of midges, which may have weakened the impact of AMF-midge interactions on aphid dynamics. Overall, aphid populations tended to decline slowest on *A.*

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curassavica plants, but fastest on *A. incarnata* plants, under medium AMF availability (Fig. 4). Our findings demonstrate that the availability of AMF in soils has complex, context-dependent effects on predator-herbivore dynamics in the field.

During our predator removal period, aphid abundances were unaffected by AMF availability. This is surprising, as AMF generally improve aphid performance (Koricheva et al., 2009), as aphids benefit from AMF-mediated increases in the size of plant vascular bundles (Simon, Wellham, Aradottir, & Gange, 2017) and may avoid AMF-mediated increases in plant defenses since phloem contains lower levels of plant secondary metabolites than leaves (Züst & Agrawal, 2016). Indeed, we found previously that AMF influence the per capita growth rates of *A. nerii* nonlinearly on these milkweed species under greenhouse conditions (Meier & Hunter, 2018b). As we reduced, but did not entirely exclude predators in this field experiment, effects of AMF on plant quality (bottom-up forces) may have been balanced by effects on mobile, generalist predators (top-down forces). For instance, mobile predators may have tracked AMF-mediated increases in aphid abundances between our predator culling days, leading there to be no apparent effect of AMF on aphids in our weekly surveys. It is also possible that *A. nerii* abundances were not affected by AMF in this experiment. Whether aphids benefit, are unaffected, or are negatively affected by AMF colonization of their host plants depends on the plant and AMF species, plant age, and developmental stage of the symbiosis (Garzo, Rizzo, Fereres, & Gomez, 2020; Koricheva et al., 2009; Meier & Hunter, 2018b; Tomczak & Müller, 2017). Indeed, in this experiment, milkweed plants were older, and aphid populations developed over a much longer time, than in our previous greenhouse experiment (Meier & Hunter, 2018b). However, because we reduced, but did not entirely exclude predators in this experiment, we cannot separate these contending mechanisms.

Our results contrast with previous findings of consistent, AMF-improved recruitment of parasitoids and predators to their herbivore prey (Babikova et al., 2013; Guerrieri et al., 2004; Hempel et al., 2009; Hoffmann, Vierheilig, Peneder, et al., 2011; Hoffmann, Vierheilig, & Schausberger, 2011a; Prieto et al., 2017; Schausberger et al., 2012; Ueda et al., 2013; Wooley & Paine, 2011). The variation in midge recruitment that we observed may have been driven by differential effects of AMF availability on the volatile blends emitted by milkweed plants and

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462 those in aphid honeydew (Meier & Hunter, 2019). Aphid midge flies are attracted to the volatiles
463 of aphid honeydew, especially benzeneacetaldehyde (Watanabe et al., 2016). In our previous
464 work, AMF did not affect benzeneacetaldehyde emissions directly, but high AMF availability
465 increased emissions of other volatiles from *A. curassavica* plants, while reducing emissions from
466 *A. incarnata* plants (Meier & Hunter, 2019). In doing so, high AMF availability reduced the
467 proportion of benzeneacetaldehyde in the overall volatile emissions from *A. curassavica* aphid
468 honeydew and plants, but increased the proportion of benzeneacetaldehyde in emissions from *A.*
469 *incarnata* aphid honeydew and plants. As the particular blend of volatiles is important in natural
470 enemy attraction (Bruce & Pickett, 2011), AMF effects on volatile blends may have driven the
471 milkweed species-specific patterns in midge recruitment. Midge recruitment is also influenced
472 by aphid location on the plant (Jandricic, Wraight, Gillespie, & Sanderson, 2013). However,
473 AMF did not affect milkweed plant size (Tables S1-S4), and *A. nerii* colonies were most dense
474 near the apical meristem of plants across treatments (A. R. Meier, personal observation). We
475 think it likely that AMF influenced midge recruitment by altering plant and honeydew volatiles,
476 not plant structure or aphid feeding location.
477
478 Once recruited to plants, the differential effects of AMF availability on the functional responses
479 of midges to aphids may have been driven by effects on aphid phenotype. While we could not
480 measure *A. nerii* sequestration in this experiment, we have found previously that *A. nerii*
481 sequester the highest concentrations of cardenolides when feeding upon plants under medium
482 AMF availability (Meier & Hunter, 2018b). Aphid midge flies are susceptible to cardenolides
483 (Mohl et al., 2020), so greater sequestration of cardenolides by aphids on medium AMF plants
484 may have reduced midge consumption of aphids with increasing aphid density (Fig. 2). In
485 contrast, aphids on high AMF plants may have been of greater nutritive quality. *Asclepias*
486 *curassavica* plants under high AMF availability had the greatest foliar phosphorous
487 concentrations (Tables S1, S2), perhaps leading midges to eat more aphids with increasing aphid
488 density on those plants (Fig. 2). *Asclepias incarnata* plants had very low levels of cardenolides
489 and their foliar phosphorous concentrations varied minimally among AMF treatments (Tables
490 S1-S4). Therefore, AMF may have had minimal effects on the phenotype of aphids feeding upon
491 *A. incarnata* plants, explaining the lack of an AMF effect on midge functional responses in *A.*
492 *incarnata*. Future work should consider how the defensive and nutritive quality of herbivores

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493 varies with AMF availability to further elucidate how AMF may shape predator functional
494 responses in the field.
495
496 Effects of AMF on the strength and magnitude of aphid mortality rates imposed by midges did
497 not strictly follow midge recruitment. These incongruities may be due to effects of AMF on
498 midge functional responses and/or intraguild predation of midges. On *A. curassavica*, midges
499 imposed their highest rates of aphid mortality on medium AMF plants, suggesting that greater
500 midge recruitment to those plants outweighed any effects of their reduced functional response.
501 However, the mortality rates of aphids imposed by midges on high AMF plants were double
502 those on plants without AMF, despite midge recruitment to *A. curassavica* plants being greater
503 on plants without AMF than under high AMF availability. The greater number of aphids killed
504 per midge on high AMF plants of *A. curassavica*, with increasing aphid density, may have
505 compensated for the reduced attraction of midges to high AMF plants. In addition, midges are
506 subject to asymmetrical intraguild predation by lacewings and coccinellids (Lucas, Coderre, &
507 Brodeur, 1998; Sentis, Lucas, & Vickery, 2012). As intraguild predation of midges is greater
508 when the ratio of midges to aphids is higher (Lucas et al., 1998), there may have been greater
509 intraguild predation of midges on plants with higher midge recruitment relative to aphid density
510 (i.e. medium AMF plants of *A. curassavica* and high AMF plants of *A. incarnata*; Fig. 1). This
511 potential reduction in midge density following recruitment may also have led to the weaker
512 effects of AMF on aphid mortality rates compared to recruitment. Future work is needed to
513 untangle AMF effects on these potential drivers of herbivore mortality rates.
514
515 Effects of AMF on the decline rate of aphid populations under full predator pressure did not
516 simply follow rates of aphid mortality imposed by midges. This incongruity was likely driven by
517 the taxon-specific effects of AMF on predator recruitment. Only midge recruitment was affected
518 by AMF availability, but lacewing, syrphid, and midge abundances were all correlated with the
519 decline rates of aphid populations. Midges may have been influenced more strongly by AMF-
520 mediated variation in plant phenotype, than were syrphids or lacewings, due to the distinct life
521 history of midges. Aphid midge flies are furtive, aphid specialists with limited mobility; larvae
522 are spatially confined to the aphid colony chosen by their mothers (Boulanger, Jandricic,
523 Bolckmans, Wäckers, & Pekas, 2019). As a result, female midge oviposition varies strongly with

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host patch quality (Jandricic et al., 2013; Mohl et al., 2020; Sentis et al., 2012). While syrphid and lacewing oviposition are also affected by host patch quality, syrphids and lacewings are relatively mobile and require over two hundred aphids for their development (Joshi & Ballal, 2013; New, 1975). Our individual experimental plants generally hosted fewer than two hundred aphids (Table S7), so lacewings and syrphids frequently colonized and dispersed from individual plants (A. R. Meier, personal observation). Therefore, lacewings and syrphids were less likely to be affected by a particular AMF treatment. Our findings demonstrate that while AMF may influence the recruitment of natural enemies with life histories tied closely to individual host plants, AMF may have weaker effects on the recruitment of mobile, generalist predators.

Indeed, most studies have found effects of AMF on the recruitment and performance of parasitoids (Babikova et al., 2013; Bennett et al., 2016; Gange et al., 2003; Guerrieri et al., 2004; Hempel et al., 2009; Wooley & Paine, 2011), whose larvae are constrained by the oviposition choice of their mothers, and polyphagous predators that also consume their host plant (Prieto et al., 2017), and are affected directly by AMF-mediated changes in plant phenotype. In one of the few other field studies performed, Ueda et al. (2013) found greater abundances of a generalist predator (*Orius sauteri*) on AMF-colonized plants in the field, but populations of these predators simply tracked AMF-mediated increases in thrip abundances. Hoffmann et al. (2011) did find greater abundances of predatory mites on AMF-colonized plants, even after accounting for AMF-mediated increases in their spider mite prey, but these predators were experimentally constrained to their host plants. Therefore, in natural settings where natural enemies can move among plants, AMF may have weak effects the recruitment of mobile, generalist predators.

Nonetheless, rates of decline in aphid populations tended to vary among AMF treatments, with the lowest decline rates on *A. curassavica* plants under medium AMF availability (Fig. 4). This resilience to population decline under full predator pressure was likely driven by AMF-mediated increases aphid sequestration of cardenolides on medium AMF plants of *A. curasssavica* (Meier & Hunter, 2018b). The survival and performance of lacewings and syrphids, like midges, are reduced substantially when they feed upon high cardenolide *A. nerii* (Malcolm, 1992; Mohl et al., 2020). Therefore, aphids feeding on *A. curassavica* plants under medium AMF availability

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may have been most toxic to predators (Meier and Hunter 2018a), leading to reduced predation and lower rates of aphid population decline on those plants.

Conclusion

In summary, we found that the availability of AMF in soils had pervasive, but variable, effects on herbivore-predator dynamics in the field. Because the availability of AMF inoculum, measured as infectivity and spore abundances, varies on small scales (Carvalho, Correia, Ryel, & Martins-Loução, 2003; Wolfe, Mummey, Rillig, & Klironomos, 2007), plants within a single population may experience substantial variation in AMF availability in soils. Based on our results, this variation in AMF abundance can result in differential recruitment of predators and predation of herbivores, generating sufficient spatial variation in trophic interactions to affect large scale population dynamics (Riolo, Rohani, & Hunter, 2015). However, the strength and direction of such effects will depend on the composition of the plant community, as AMF effects on predator-prey dynamics were highly plant species-specific. Furthermore, our study was limited to a single AMF species, although plants are colonized naturally by a diversity of AMF taxa simultaneously (Smith & Read, 2008). AMF taxa, and combinations of taxa, have distinct effects on plant phenotype (Bennett, Bever, & Bowers, 2009; Thonar, Frossard, Šmilauer, & Jansa, 2014), and resulting herbivore performance (Barber, Kiers, Hazzard, & Adler, 2013; Roger, Gétaz, Rasmann, & Sanders, 2013). Therefore, future work should also consider how variation in abundances of natural AMF communities shapes predator-prey interactions aboveground.

Furthermore, we found that effects of AMF on predator recruitment are predator taxon-specific. Therefore, how AMF ultimately shape multitrophic interactions will depend on effects on communities of natural enemies, not individual natural enemy species. For instance, AMF may affect multitrophic interactions strongly if the dominant natural enemies are specialists or constrained to an individual host plant for much of their development (e.g. parasitoids, aphid midge flies), but AMF may have weaker effects if mobile, generalist predators are the primary top-down forces acting on herbivore populations. To further elucidate the mechanisms by which AMF shape multitrophic interactions in natural systems, future studies should evaluate how natural AMF abundances influence the strength of bottom-up and top-down forces acting on

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herbivore populations, with explicit consideration for communities of natural enemies and interactions within those communities.

Data accessibility: Data are available from the Dryad Digital Repository: https://doi:10.5061/dryad.qv9s4mwdc (Meier and Hunter, 2021).

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Authors' contributions: ARM and MDH conceived the ideas and designed methodology; ARM collected the data; ARM and MDH analyzed the data; ARM led the writing of the manuscript. Both authors contributed to the drafts and gave final approval for publication.

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Tables and Figures

Table 1. F-values, degrees of freedom, and P-values of a generalized linear mixed model analysis, with a negative binomial distribution and log link function, of the effects of the availability of arbuscular mycorrhizal fungi (AMF) inoculum, milkweed species, aphid abundance, and their interactions on the recruitment of midge eggs and larvae (combined) over time (Weeks 2-6). N=10-16 per treatment combination.

Factor	**F**	**P**
AMF	$F_{2,6}$=2.54	0.1591
Plant Species	$F_{1,3}$=3.97	0.1404

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AMF x Plant Species	$F_{2,5}$=7.85	0.0287
Weeks	$F_{4,12}$=8.8	0.0015
AMF x Weeks	$F_{8,24}$=0.53	0.8205
Plant Species x Weeks	$F_{4,12}$=4.5	0.0189
AMF x Plant Species x Weeks	$F_{8,19}$=3.17	0.0185
Aphids	$F_{1,321}$=134.45	<0.0001
Aphids x AMF	$F_{2,321}$=1.57	0.2089
Aphids x Plant Species	$F_{1,321}$=5.02	0.0258
Aphids x AMF x Plant Species	$F_{2,321}$=11.07	<0.0001
Aphids x Weeks	$F_{4,321}$=3.69	0.0059
Aphids x AMF x Weeks	$F_{8,321}$=0.76	0.6427
Aphids x Plant Species x Weeks	$F_{4,321}$=1.86	0.1176
Aphids x AMF x Plant Species x Weeks	$F_{8,321}$=3.77	0.0003

Table 2. F-values, degrees of freedom, and P-values from analyses of the effects of the availability of arbuscular mycorrhizal fungi (AMF) inoculum, milkweed species, aphid abundances in Week 6, and their interactions on the number of aphids killed per midge and number of aphids killed by midges in Week 7. Aphids killed per midge was analyzed using a linear mixed model, and the number of aphids killed by midges was analyzed using generalized linear mixed model with a negative binomial distribution and log link function. N=10-16 per treatment combination.

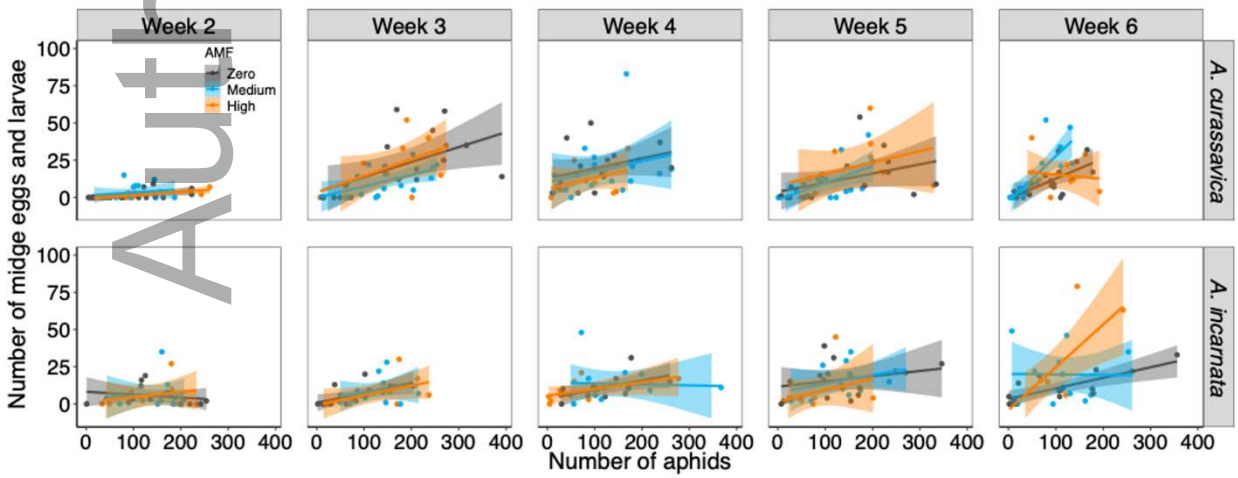
	Number of aphids killed per midge		**Number of aphids killed by midges**	
Factor	**F**	**P**	**F**	**P**

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AMF	$F_{2,47.9}$=1.36	0.2671	$F_{2,62}$=2.16	0.1245
Plant Species	$F_{1,46.9}$=0.00	0.9532	$F_{1,62}$=0.25	0.6213
Plant Species x AMF	$F_{2,48.2}$=1.96	0.1515	$F_{2,62}$=2.99	0.0576
Aphids	$F_{1,48.6}$=5.53	0.0227	$F_{1,62}$=29.55	<0.0001
AMF x Aphids	$F_{2,47.3}$=0.95	0.3947	$F_{2,62}$=1.64	0.2016
Plant Species x Aphids	$F_{1,47.8}$=1.01	0.3208	$F_{1,62}$=0.02	0.8876
Plant Species x AMF x Aphids	$F_{2,48}$=2.87	0.0665	$F_{2,62}$=3.02	0.0562

Figure 1. Effects of the availability of AMF inoculum on the number of midges (eggs and larvae) recruited to *A. curassavica* and *A. incarnata* plants, relative to aphid density, during the weeks of reduced predator pressure. Each point represents an individual plant, and the shaded regions represent 95% confidence intervals around the curves. N=10-16 per treatment combination.

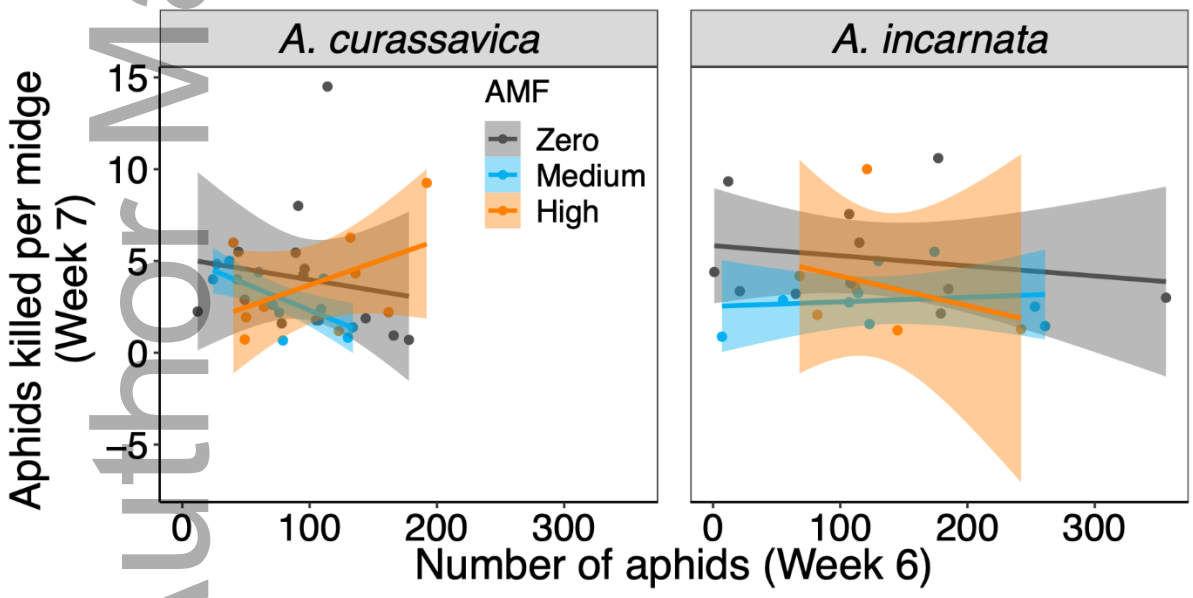


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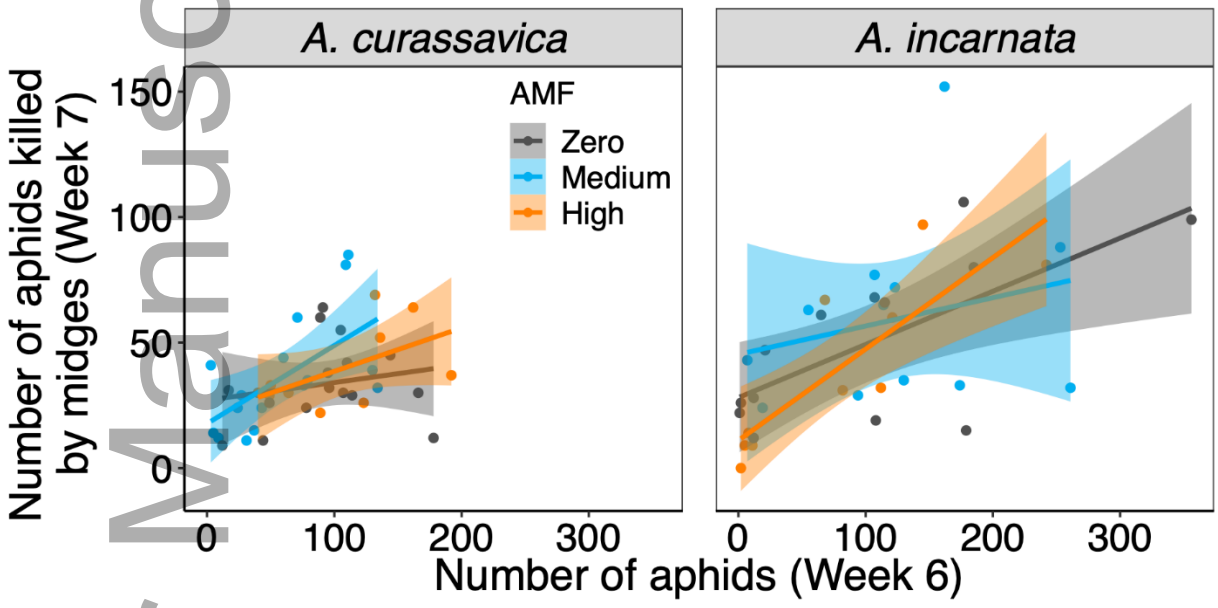
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Figure 2. Effects of the availability of AMF inoculum on the number of aphids killed per midge (Week 7), relative to density of aphids the previous week (Week 6), on *A. curassavica* and *A. incarnata* plants. Each point represents an individual plant, and the shaded regions represent 95% confidence intervals around the curves. N=10-16 per treatment combination.



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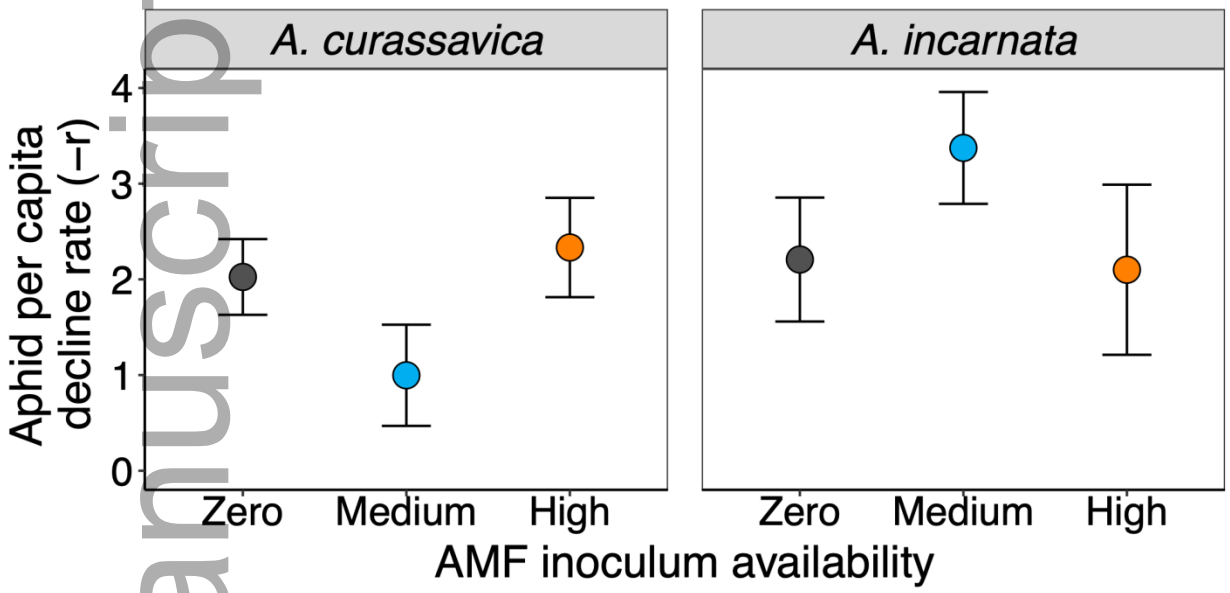
Figure 3. Effects of AMF availability on the number of aphids killed by midge larvae (Week 7) relative to the number of live aphids present in the previous week (Week 6) on *A. curassavica* and *A. incarnata* plants. Each point represents an individual plant, and the shaded regions represent 95% confidence intervals around the curves. N=10-16 per treatment combination.



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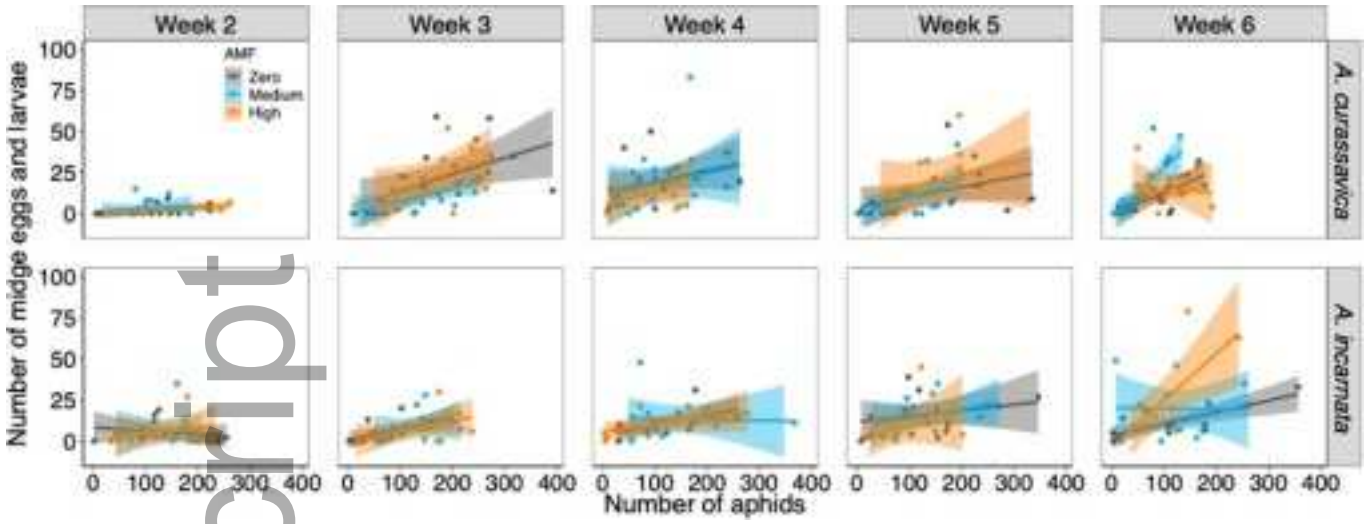
869 **Figure 4.** Effects of AMF availability on the rate of aphid population decline under full predator
870 pressure (-r; -ln(aphid population Week 7+1/aphid population Week 6) on *A. curassavica* and *A.*
871 *incarnata*. Points represent treatment means ± 1 SE. N=10-16 per treatment combination.
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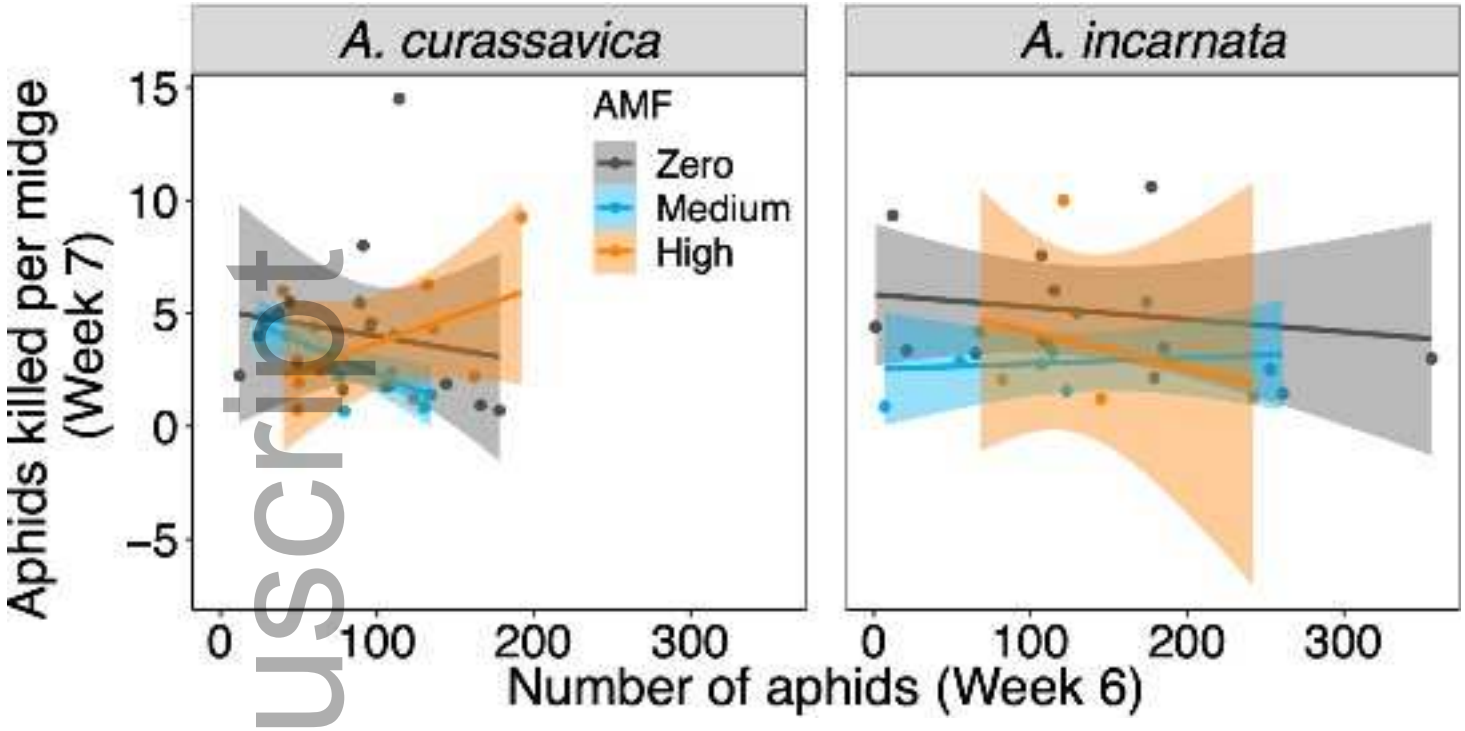
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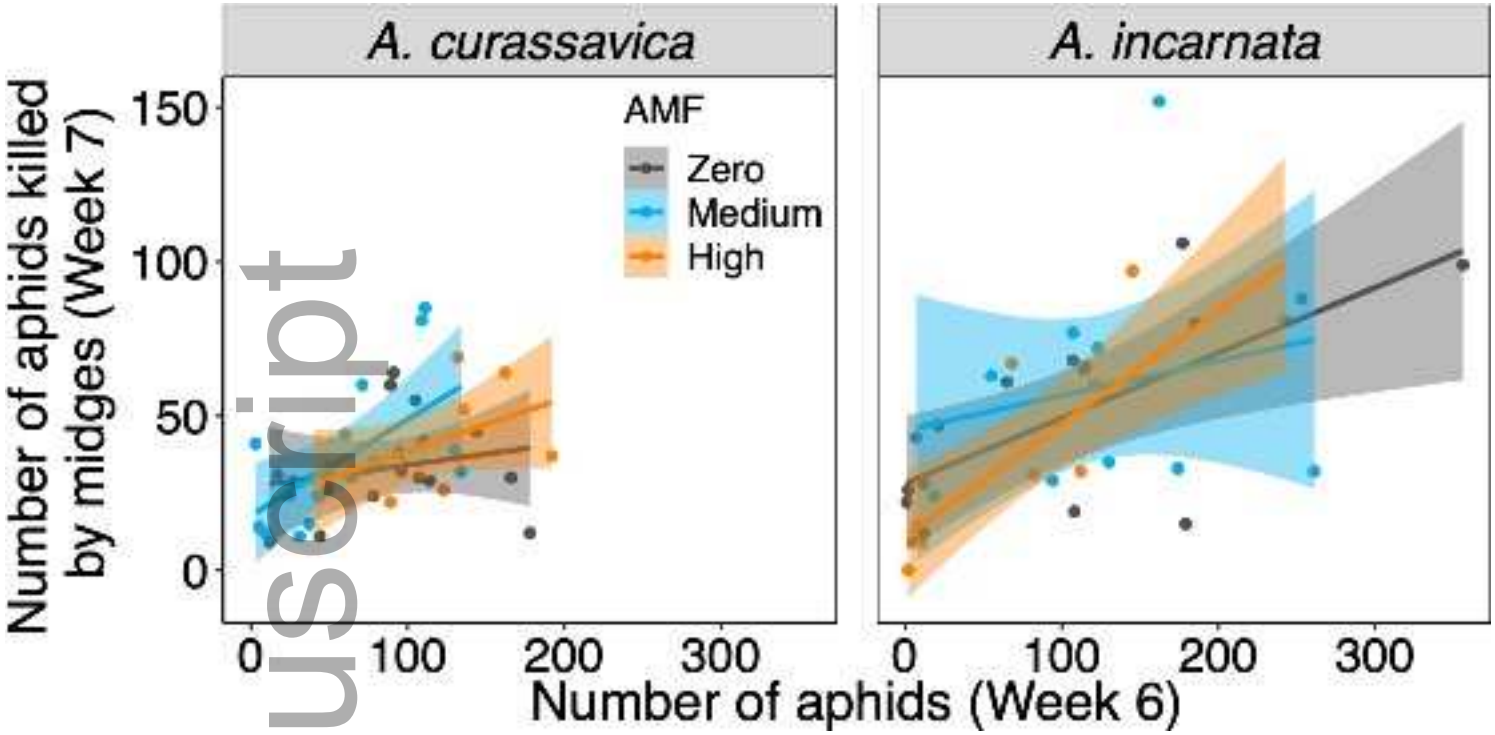
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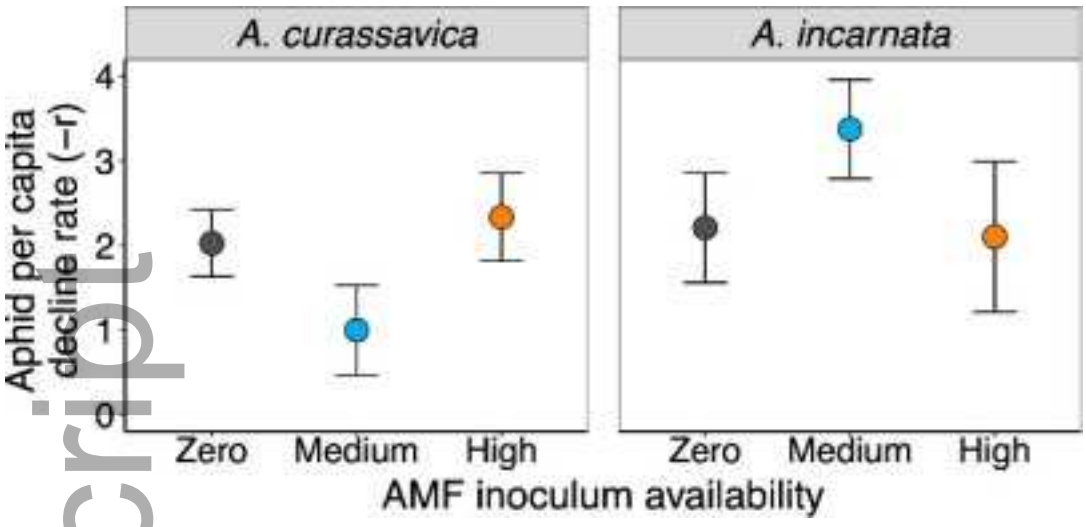
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