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FELLOWSHIP FINAL REPORT

How do soil microbes influence plant attraction of insect herbivores and/or parasitoids of herbivores?

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REPORT INFO

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ABSTRACT

Plants are exposed to multiple stressors simultaneously, but can receive help deterring biotic stressors from belowground mutualists such as arbuscular mycorrhizal fungi. Previous research has identified that this "help" occurs primarily via enhanced direct defences against chewing herbivores. However, our preliminary data had suggested that AM fungi promote indirect defenses against sucking herbivores like aphids. Here we tested this premise in tomato. We exposed plants to colonization by AM fungi or not, and herbivory by potato aphids or not. We then measured plant biomass as well as changes in volatile organic chemistry over time, and attraction to plants within our treatments by parasitoids in wind tunnel trials. While analyses are ongoing, we found an influence of AM fungi on plant biomass and a trend toward greater attraction of parasitoids to plants hosting AM fungi. Surprisingly we found no impact of aphid herbivores on the attraction of their parasitoids, and no interaction between AM fungi and aphid herbivory on parasitoid attraction. This suggests that AM fungi do, as we hypothesized, promote indirect defences of plants.

1- Introduction

Plants are faced with a myriad of enemies, and, unlike many animals, they cannot run away from their predators (herbivores). However, plants don't face these enemies alone—they get a bit of help from beneficial microbes in the soil. The beneficial microbes arbuscular mycorrhizal (AM) fungi benefit their plant hosts in multiple ways: they aid uptake of important nutrients and water, promote tolerance to abiotic stresses such as drought, and prime plants for faster immune responses against pathogens and herbivores [1]. These fungi are obligate partners of plants, and live-in roots and soil, foraging for nutrients that can be transported along their hyphal network and delivered to plants.

The plant immune system allows recognition of potential antagonists and activation of defense responses and is controlled by complex regulatory networks that integrate responses to both internal and external cues. Here we focus on plant direct defenses, and specifically induced defenses (as immune responses do not influence constitutive defenses). The recognition of cues by plants and the activation of signaling cascades allows them to prioritize immune responses and regulate their growth–defense balance [2], as well as transcriptionally reprogram their metabolome to efficiently cope with diverse threats [3]. For example, jasmonate mediated signaling regulates responses to wounding by

insect herbivory and necrotrophic fungal pathogen infection [4].

Work on chewing herbivores that respond to jasmonate mediated signaling has demonstrated that priming impacts both direct and indirect defenses in plants. Direct defenses directly deter herbivores and include changes in plant secondary metabolite concentrations, leaf toughness, and surface trichomes. Indirect defenses lead to recruitment of insect enemies of herbivores via the release of volatile metabolites into the air that are detected by herbivore enemies. Early work demonstrated that AM fungi prime direct defenses in ways that impacted chewing herbivores more strongly than sucking herbivores [5]. Work on indirect defense has also shown that volatile composition changes following AM fungal colonization and herbivory by chewing herbivores [6] The majority of work on priming by AM fungi has been conducted on tomato in the group of collaborator Dr. Maria Pozo (CSIC, ES) [7-9], but priming has also been shown to occur in potato [10].

However, the focus of this research field on chewing herbivores has prevented us from understanding potential impacts of priming on sap-feeding herbivores like aphids. Dr. Ali Karley (James Hutton Institute, UK) and I have shown that AM fungi, plant species and aphid clonal line strongly influence the performance of aphids [11-14]. In addition we have also demonstrated that AM fungal communities alter aphid parasitoid success on potato plants [e.g.,11, 12-14], and this has led us to hypothesize that AM fungi promote indirect defense (calling to herbivore enemies or promoting herbivore enemy fitness) when fed on by sap-feeding herbivores. Here we address the question of whether AM fungi alter aphid feeding and parasitoid attraction through plant physiological changes.

2- Experimental details

To address this gap we conducted a fully factorial experiment growing tomato (*Solanum lycopersicon* cv. Moneymaker) with and without *R.irregularis* MUCL57021 (REKA B.V., Bleiswijk, NL) and manipulating the presence of herbivory by potato aphids (*Macrosiphum euphorbiae*).

The *Macrosiphum euphorbiae* aphid colony used in this study was started from a single virginiparous female which originated from Torrelodones (Madrid, Spain) and was collected on tomato and maintained on tomato cv. Marmande. The colony was reared at the Insect Vectors of Plant Pathogens laboratory of the Institute of Agricultural Sciences-Spanish National Research Council (ICA-CSIC in Madrid, Spain). The colony was maintained in a growth chamber at 23:18°C (day: night), 75% relative humidity, and a photoperiod of 14:10 h (light: dark). Three days prior to placing insects on the plants, aphid nymphs were sent to IRBI in France via FedEx.

Background soil for the experiment consisted of 1 part mineral garden soil with 18% organic matter mixed with 2 parts sand. Soil was steam sterilized for two hours twice.

Aphidus ervi parasitoids were ordered from X () as eggs, placed in an insect cage/bug dorm immediately. Three days after arrival, parasitoids began to emerge and were removed daily to the best of our capability.

Plants were germinated in sterile potting soil and three weeks after germination seedlings were transplanted into 1 L pots containing background soil inoculated with live or sterile *R. irregularis*. In each pot 200ml of sterile background soil was added followed by 500ml of sterile background soil mixed with 100ml of live or sterile inocula followed by 200 ml of sterile background soil. This design ensured plants encountered AM fungal inocula and minimized contamination between pots. Plants were divided into two blocks and plant of different treatments were randomly assigned within each block. Plants were initially established in a greenhouse, but transferred to a growth chamber after two weeks (settings: 12 hours of daylight, regular humidity, 21C). Plants were watered by hand regularly and

fertilized every two weeks with 25 ml of 20:0:20 NPK fertilizer (3g fertilizer/1000ml). After one month plants were also fertilized with 10ml of Bone & Muscle Calcium & Magnesium Superconcentrated Fertilizer (Madame Grow, Sevilla, ES) (diluted to 3ml/1000ml) as plants were showing signs of Calcium deficiency.

After four weeks of growth, we bagged plants with plastic bags left open at the top to allow respiration, and after five weeks of growth we added 1 aphid nymph to bagged plants in the herbivory treatment. At this time, we also placed Solid Phase Microextraction (SPME) fibers in the bags and closed the bag to allow passive collection of volatile organic compounds. After one hour we removed SPME fibers and opened the bags. We recorded aphid presence and sampled with SPME fibers on day 4, day 8, and day 11 following aphid addition. Volatiles were extracted from SPME fibers and analyzed using GC-MS.

Between days 8 and 11 we conducted wind tunnel trials to assess whether AM fungal and herbivory treatments influenced attraction of the aphid parasitoid *Aphidus ervi* to plants. Plants were placed in a mesh insect cage and placed between 90 cm and 110 cm away from the release chamber of the wind tunnel. Wind flow in the tunnel was 7.3 bar, and 10 parasitoids were placed in the release chamber prior removing the wall blocking access to the remainder of the tunnel. Trials lasted 10 minutes and the location of the parasitoids was recorded after 5 minutes in most of the trials and after 10 minutes. Between trials the previous plant was removed and the chamber was aired for five minutes before adding the next plant and the next set of aphids.

Aphids were removed after SPME sampling on day 11, and aboveground and belowground tissues were harvested. Samples to assess AM fungal root colonization (average 1g of wet weight) were taken during the belowground harvest and aboveground and the remaining belowground biomass were dried and weighed.

Data analysis is ongoing. Dry weight of above and belowground biomass, number of flowers, number of aphids per plant, root colonization (data pending), and data from the wind tunnel assays (proportion of parasitoids that traveled the length of the chamber to reach plant in the bug dorm, proportion of parasitoids that never entered the chamber during the trial, proportion of parasitoids that entered the chamber after 5 minutes, and distance traveled by parasitoids in the chamber) were analyzed as dependent variables in a general linear model in SAS using the glm procedure. Independent variables included block, presence of AM fungi, presence of aphid herbivory, and the interaction between these two treatments. Data analysis of the volatile organic chemistry is forthcoming.

3- Results and discussion

AM fungal treatment significantly influenced plant biomass as plants from the sterile treatment had greater root, shoot, and total biomass. There was a trend for more parasitoids to enter the wind tunnel if plants hosted AM fungi ($F_{1,19} = 4.08$, $p = 0.058$). Our results show a clear negative impact of AM fungal inoculation on plant biomass, and a likely positive impact on the attraction of parasitoids to plants that host AM fungi. Surprisingly, in the data analyzed to date, we see no clear impact of aphid herbivory on attraction of parasitoids to host plants. This suggests that AM fungal modify host plant volatiles in a way that more strongly attracts parasitoids than a relatively low (two aphid nymphs) herbivory colonization. This may benefit host plants or cause confusion on the part of parasitoids that reduces efficacy of attraction when plants face greater herbivore pressure. We expect the analyses of volatile organic chemicals to provide greater detail surrounding the impacts of AM fungi on herbivore attraction.

4- Conclusion

While analyses are still ongoing, we saw an impact of AM fungi on plant biomass and parasitoid attraction to plants hosting AM fungi.

5- Perspectives of future collaborations with the host laboratory

The volatile organic chemical analyses were conducted at IRBI but are still ongoing. As a result, we will continue to collaborate to complete those analyses. In addition, we feel our trends would be significant results if we were able to have higher replication. As a result, we have discussed repeating the experiment with a greater number of plants.

6- Articles published in the framework of the fellowship

We expect to publish at least one paper highlighting the results of this project

7- Acknowledgements

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