THE GALL TO GROW: EFFECTS OF GALLS ON FUNGAL GROWTH AND ARTHROPOD ABUNDANCE

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Abstract

Manipulation of oak apple galls by the California gall wasp (*Andricus quercuscalifornicus*) has been shown to have long-lasting effects on arthropod communities in valley oak (*Quercus lobata*) foliage. The California gall wasp acts as an important ecosystem engineer that indirectly affects resource availability to nearby organisms. We explore the possible effects galls may have at the ground level as they fall to the soil. Two lab experiments, one testing *Agaricus bisporus* growth and the other testing growth of three species on agar media, reveal that gall presence can have significant effects on fungal growth. The mechanism is unknown, but the high tannin content of galls provides a possible explanation. A field experiment testing for insect abundance revealed a negative relationship between mycelial colonization and arthropod abundance. Further research in this area would help to explain relationships between fungi and arthropods and the mechanisms to fungal growth inhibition.

Keywords: gall, cynipid gall wasp, fungi, mycelium, growth, arthropod

Introduction

Manipulation of oak apple galls by the California gall wasp (*Andricus quercuscalifornicus*) has been shown to have long-lasting effects on arthropod communities in valley oak (*Quercus lobata*) foliage (Wetzel et al., 2014). In a field experiment performed by Wetzel et al. (2014), galls were found to be colonized by secondary inhabitants such as jumping spiders who used emergence tunnels left over by gall wasps. They then found through gall

removal that these jumping spiders likely influenced the arthropod community by inhabiting galls, with gall removed trees containing 60% more herbivores and 30% more arthropods. This experiment demonstrated that the California gall wasp acts as an important ecosystem engineer, which is an organism that "directly or indirectly controls the availability of resources to other organisms by causing physical state changes in biotic or abiotic materials" (Jones et al., 1997). Furthermore, ecosystem engineers tend to be more important when an ecosystem has persistent structuring (Jones et al., 1997), exemplified by gall wasps who leave galls that persist for several years on branches and become available for secondary inhabitants. With gall wasps having large indirect effects on arthropod communities in valley oak foliage, the possibility exists that there are other indirect effects occurring at ground level beneath valley oak trees with galls once they fall to the ground.

At ground level, fungi are present as a resource or a habitat for arthropods. Studies have found that fungi are important to the survival of many arthropod species. For instance, in a survey of 14 polypore fungal species in Chicago, around 90 genera and 70 species of arthropods were identified, with the orders Acarina, Collembola, and Coleoptera being some of the dominant orders present (Graves, 1960). Of these orders, Collembola is also known to contain species of soil-dwellers who consume mycelium, and another arthropod known to consume mycelium is the mite (Shaw, 1992). Therefore, changes in fungal growth in an ecosystem could possibly lead to changes in arthropod abundance and/or diversity by affecting resource or habitat availability.

Change in fungal growth may indeed be a factor in valley oak ecosystems, possibly because of the unusually high tannin content of galls. An analysis of *Quercus robor L.* apple galls found an estimated tannin content of 47.2% (Paaver et al., 2010). These tannin levels are

then concentrated in the outer gall tissue as cynipid gall wasps manipulate oak tree hosts for nutritive purposes. Tannin levels have been found to be lower in inner gall nutritive tissue where cynipid larvae thrive, and significantly higher in the outer gall tissues of two oak species (Ikai & Hijii, 2007). In addition, previous studies have found an inhibitory effect of oak leaf litter tannins on different species of fungi, leaving the possibility that gall tannins could have a similar effect. An experiment performed by A.F. Harrison in 1970 tested for effects of leaf litter tannins on 19 different species of fungi, and inhibition was discovered in 14 of the species. 6 of these species were identified as soil fungi, indicating that tannins from leaf litter could have an effect on organisms growing in soil (Harrison, 1971). A similar environment can be created under oak trees containing galls, where galls falling to the ground can create a layer above the soil.

Here, we explore the effects of gall presence on the growth of the button mushroom *Agaricus bisporus* in the lab and the subsequent effects on arthropod abundance in an oak savannah setting. First, we hypothesize that the button mushroom *Agaricus bisporus* (hereafter *Agaricus*) will show decreased mycelial colonization and pinning with increasing percentages of gall in compost. Second, we hypothesize that three species of fungi plated in agar media containing gall material will show reduced mycelial growth rates. Lastly, we hypothesize that *Agaricus* grown in low-gall conditions will display a greater degree of mycelial colonization, and will ultimately increase the arthropod abundance in compost.

Methods

Colonization and Pinning

We performed a lab experiment by growing *Agaricus bisporus* to observe the effects of oak apple gall material on *Agaricus* colonization and pinning. We obtained compost and *Agaricus* grain spawn from Monterey Mushroom Inc. (Watsonville, CA), and gathered oak apple

galls from an oak savannah at the Russell Ranch Wildlife Area in Davis, CA, USA (lat 38.539, long -121.867). Galls were dried in an oven at 70° C for 2 hours and then grounded into a powder-like dust. Next, three treatments were formed using compost and the following percentages of gall dust by volume: 0%, 10%, and 40%. Treatments with gall were hand mixed until they reached uniform distribution, and water was added to retain moisture. We gathered 180 2.5 gallon pots and lined them with clear plastic bags. Three gallons of a compost treatment was added to each replicate, resulting in 60 replicates of each treatment. 200 mL of spawn was added to each replicate and the mixture was hand mixed until it was uniformly distributed. Plastic bags were rolled to prevent air flow, however bags were aired out every 2 days for air flushing. After 1 month, a casing layer was added to each replicate. Large mixes were formed using 66.2 L peat moss, 40 L water, and 4 L lime. 2 L of this mixture was added on top of the compost in each replicate. After 2 months since the initial addition of spawn, colonization was measured from a scale of 0 to 5 (linear scale where 0 = no growth and 5 = maximum mycelial growth). Pinning, the first formation of the mushroom fruiting body (Beyer), was measured on a scale of 1 to 5 by observing the number of pins in the casing layer (1 = no colonization, 2 = colonization, 0 pins, 3 = 1-5 pins, 4 = 5-10 pins, 5 = >10 pins).

Plating Experiment

Another lab experiment was performed by plating *Agaricus bisporus*, *Pleurotus ostreatus*, and *Trichoderma viride* species on agar mixes (hereafter *Pleurotus* and *Trichoderma*). These species were chosen to cover a wide range of life histories: *Agaricus* is a secondary decomposer, *Trichoderma* is a fast-growing primary decomposer, and *Pleurotus* is a slower-growing primary decomposer. We created two agar mixes: one containing 20 g gall/L PDA and the other being a 0 g gall/L control. PDA media solution was composed of 39 g potato dextrose

agar per L of media. All media was autoclaved to prevent contamination by bacteria or mold and poured into 10 cm diameter Petri dishes (hereafter plates). Pure cultures of each species were obtained. We inoculated plates by cutting an agar cube off the growing edge of the pure culture and placing it in the center of a new plate. This was performed using sterile tools under a Laminar flow hood. Each species was plated on 10 plates of 0 g gall/L agar and 10 plates of 20 g gall/L agar, resulting in a total of 60 plates. Growth was measured by lining a ruler with the agar cube and measuring the diameter in cm of mycelial growth. The diameter perpendicular to the first measurement was also recorded, and the two values were averaged. Number of hours since inoculation was recorded in order to calculate growth rate in units of cm/day. To determine whether growth was possible on gall dust alone, we plated each species on 2.5 g gall dust mixed with 20 mL deionized water with 3 different treatments of gall dust: autoclaved, baked (in oven at 70° C for 2 hours), and unbaked. Each species was plated on 10 plates for each treatment for a total of 90 plates.

Arthropod Survey

The replicates of *Agaricus* were then taken out to perform a field experiment at Russell Ranch. Trees in the oak savannah were randomly chosen for analysis, and 25 “No Gall” trees and 25 “High Gall” trees were chosen. “High Gall” trees were defined as trees with 100 galls or more. Previously to bringing replicates to the field, we measured water content and weight of each replicate. Gravimetric water content was assessed by weighing 1 tablespoon of compost wet, and then re-weighing after it had been dried for 24 hours at 70° C. Lastly, bags were weighed. Each tree at the field site was divided into 8 pie-shaped octants, and 3 octants were randomly chosen to be used for *Agaricus* placement. 1 of each of a 0%, 10%, and 40% replicate were randomly assigned to the 3 octants. The *Agaricus* compost piles were removed from their

bags and placed 1 m from the base of tree in the middle of the octant. After 5 days, we returned to the field site to bring samples back to the lab. We took a knife, looked down at the *Agaricus* mix, cut 1 half minus 1 knife length of a randomly chosen side, and placed this sample in a bag. In the lab, 500 mL of this sample was used to perform a floating analysis to assay invertebrate communities. This sample was added to a mixture of 80 mL of sugar and 1000 mL of water. Before adding the sample to water, 2 minutes was spent looking for arthropods in bags. After adding the sample to water, 4 minutes was spent with the float under the microscope to look for nematodes and microscopic organisms. Then, 16 minutes was spent looking at floats at eye level. Arthropods found were removed and stored in vials with ethanol, and arthropod abundance data was recorded.

Statistical analysis

Statistical analysis was performed using R (R Core Team, 2014) in R Studio (RStudio, 2015). We ran an ANOVA followed by a Tukey Honest Significant Difference Test on the variables colonization and compost treatment. It should be noted that data was not normally distributed according to the Shapiro test, which does not meet one of the assumptions of the model. The same tests were ran on the variables pinning and compost treatment. To test the effects of colonization on abundance of certain orders of arthropods, I ran multiple nested ANOVAs with the tree as a nested variable. I then ran a nested ANOVA on total number of arthropods found (in floats and pre-floats combined) and *Agaricus* colonization, taking the tree into consideration. I ran a test using the full dataset and a test excluding a unique data point which contained a total of 2002 arthropods, which was 1740% greater than the second highest data point. I present results without this data point as this one data point had a disproportionate effect on results. Lastly, regressions were ran with covariates including gravimetric water content

and weight of the compost pile to determine whether these had an effect on arthropod abundance. To test for an effect of gall level on *Trichoderma* growth rate, I ran a t-test on the variables growth rate and g gall/L in agar.

Results

Agaricus colonization levels differed significantly among gall percentage in compost. Significant differences were found between all compost treatments, with a mean difference of 0.84 between 0% and 10%, a mean difference of 2.16 between 0% and 40%, and a mean difference of 1.32 between 10% and 40% (Figure 1, Table 1). *Agaricus* pinning levels differed significantly between 0% and 40% treatments with a mean difference of 0.74, although the difference was only marginally significant between 10% and 40% and not significant between 0% and 10% (Table 2).

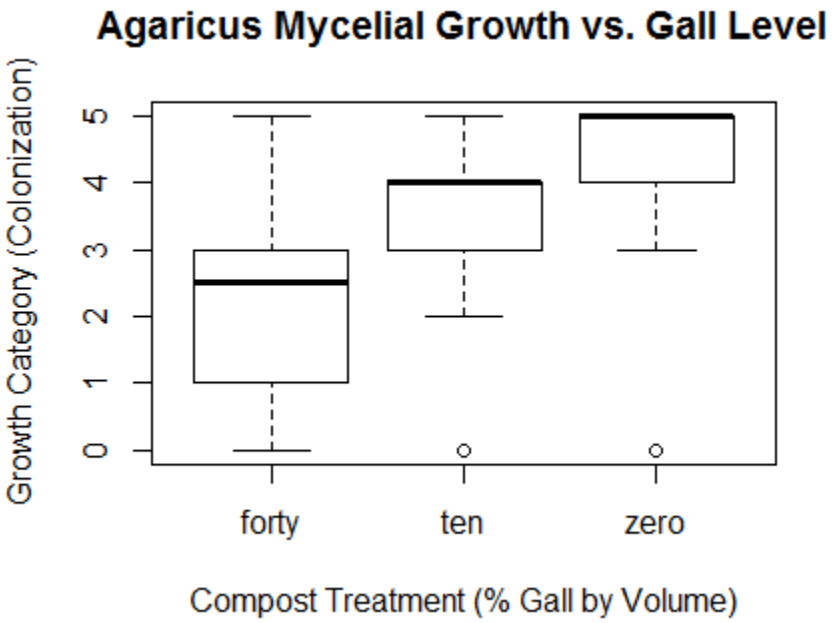

Figure 1. Average differences in *Agaricus bisporus* mycelial colonization among three treatments of compost. All groups differed at the a = 0.05 level.

Table 1. Results of Tukey's Honest Significant Difference on an ANOVA comparing differences in *Agaricus bisporus* colonization among three treatments of compost. Differences to the a = 0.05 level were observed between the 0 – 10%, 0 – 40%, and 10 – 40% treatments.

Treatment	Mean Difference	Lower CI	Upper CI	p-value
0 – 10%	**0.84**	**0.32**	**1.36**	**0.0005**
0 – 40%	**2.16**	**1.64**	**2.68**	**<0.0001**
10 – 40%	**1.32**	**0.80**	**1.84**	**<0.0001**

Table 2. Results of Tukey's Honest Significant Difference on an ANOVA comparing differences in *Agaricus bisporus* pinning among three treatments of compost. Differences to the a = 0.05 level are highlighted in bold.

Treatment	Mean Difference	Lower CI	Upper CI	p-value
0 – 10%	0.30	-0.17	0.77	0.303
0 – 40%	**0.74**	**0.26**	**1.22**	**0.001**
10 – 40%	0.44	-0.03	0.92	0.079

Gall presence in agar media influenced mycelial growth of the three species tested. *Agaricus* and *Pleurotus* grew on media with 0 g gall/L, but were not observed to grow on media with 20 g gall/L (Table 3). *Trichoderma* was capable of growth on both media treatments, however its growth rate was significantly higher on media without gall: 1.07 cm/day on 0 g gall/L while being only 1.03 cm/day on 20 g gall/L (Figure 2, t=2.9785, n_1=10, n_2=10, P=0.008). In the gall dust experiment, *Trichoderma* and *Pleurotus* grew on autoclaved gall dust, but the two species could not grow on baked or unbaked treatments (Table 3). *Agaricus* did not grow on any of the 3 gall dust treatments (Table 3).

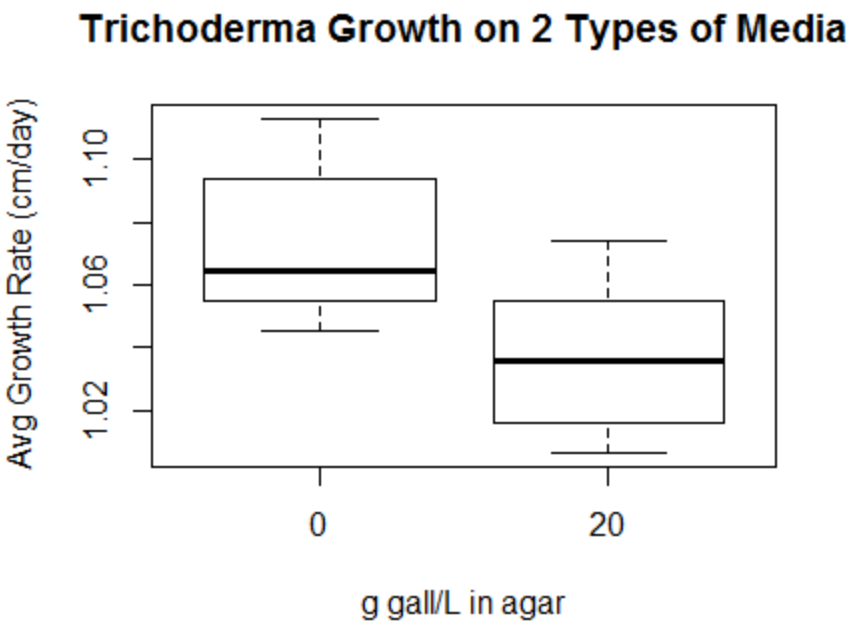

Figure 2. *Trichoderma* growth rates on 0 g gall/L agar and 20 g gall/L agar. Growth rate was significantly different among the treatments (t = 2.9785, n_1 = 10, n_2 = 10, p = 0.008).

Table 3. Summary of visible growth over both the agar and gall dust experiments. Visible growth was measured at human eye level.

Treatment	***Trichoderma***	***Pleurotus***	***Agaricus***
0 g gall/L	Yes	Yes	Yes
20 g gall/L	Yes	No	No
Autoclaved dust	Yes	Yes	No
Baked dust	No	No	No
Unbaked dust	No	No	No

A weak, marginally significant trend was found when testing the effects of mycelial colonization on arthropod abundance (F = 7.365, df = 1, p = 0.07). On average, arthropod abundance was higher at lower levels of colonization (Figure 3). When testing effects of colonization on abundance of an order, such as flies or beetles, no significant relationships were found. The tree did not have an effect on any results. Covariates including gravimetric water content and pot weight did not have significant effects on arthropod abundance.

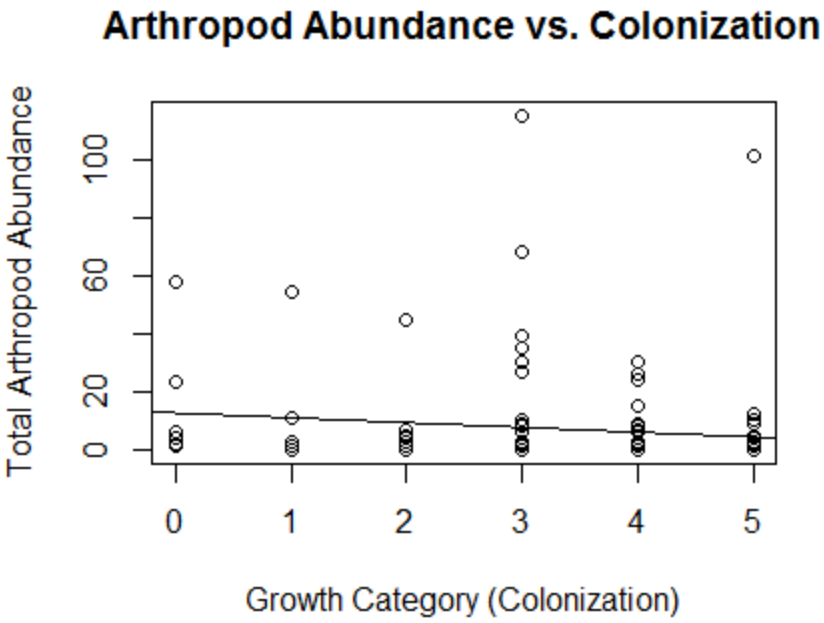

Figure 3. Effect of colonization level on arthropod abundance. A marginally significant trend was found (F = 7.365, df = 1, p = 0.07), displaying a negative relationship.

Discussion

Compost containing a larger percentage of oak apple gall appeared to cause inhibition of *Agaricus* mycelial colonization in compost, suggesting that the chemical components of galls may have a negative effect on fungal growth. As discussed previously, tannins are likely the chemical with the greatest effect considering the large concentrations of tannins in the outer gall tissue. These results are supported by the results from the plating experiment, which show decreased mycelial growth for *Trichoderma* and lack of mycelial growth for *Agaricus* and *Pleurotus* in the 20 g gall/L agar treatment compared to the 0 g gall/L control. Interestingly, the gall dust portion of the plating experiment revealed that growth was possible on autoclaved gall dust for *Trichoderma* and *Pleurotus*, however, growth was not observed for any species on non-autoclaved gall dust. One possible explanation is that the autoclave process produced enough

heat to deactivate a significant portion of tannins, resulting in a more tolerable environment for fungi. A study which involved autoclaving of faba beans containing tannins found that "more than half of the response to heat treatment is associated with the inactivation of tannins" (Brufau et al., 1998). If the autoclave was indeed deactivating tannins, this could mean the 20 g gall/L agar mix actually had a lower amount of activated tannins than in a naturally occurring gall. Despite this, the agar mix still had an inhibitory effect, suggesting that an adequate amount of tannins remained activated in the agar mix. There also exists the possibility that the 20 g gall/L agar mix had a physical effect with gall material impeding growth with its presence rather than a chemical effect. However, considering that growth was possible on autoclaved dust, the physical structure of the substrate appears to have little or no effect on growth. Further tests on the chemical components of gall dust before and after autoclaving would help to confirm which hypothesis is more likely.

Arthropod surveys produced opposite results to what we hypothesized. Instead of compost piles with greater degrees of colonization attracting more arthropods, on average, fewer arthropods were found in highly colonized compost. Originally, we predicted that greater mycelial biomass would attract more arthropods, with the assumption that certain arthropods would benefit from having mycelium as a plentiful resource. Instead, it is possible that availability of burrowing space influenced whether arthropods entered the compost piles. Since no relationships were found between colonization and abundance of any arthropod orders, we likely did not attract specialists that rely on mycelium as a primary resource. In this case, greater colonization could make it more difficult for generalist arthropods to burrow into the compost and use the soil as a resource. One factor that was not considered is that fruiting bodies of mushrooms could hold greater importance to arthropods as they offer a refuge for some species

to deposit larvae. Fruiting bodies could act as shelter and as an easily accessible food source for larvae simultaneously, and they can even increase larvae survivability as seen in species of *Drosophila* (Grimaldi & Jaenike, 1984). Although mycelium contains some nutritive value (Miles & Chang, 2004), it likely does not offer the same protective qualities that a fruiting body can offer. A repeat of this experiment would likely benefit from growing *Agaricus* to a fruiting body stage. This change would paint a clearer picture of which species are directly burrowing inside fungi, and it could also answer whether inhibition to mycelial colonization has an effect on fruiting body nutritional quality. If gall inhibition on colonization leads to poorer quality of fruiting body, resource availability to arthropods could be affected.

Conclusion

Although we did not find strong evidence that arthropod abundance is affected by fungal growth, the possibility remains that fungal growth could have an effect on arthropod abundance. We demonstrate that galls appear to inhibit fungal growth in two separate experiments, with tannins being likely culprit. In some circumstances, this may benefit generalist arthropods by decreasing underground mycelial growth and providing space for burrowing. Further research would clarify whether specialist arthropods, especially those who rely on fungal fruiting bodies, are indirectly affected by gall presence. The literature supports the possibility that specialists on fungi could be affected by resource availability provided by fruiting bodies. Overall, this paper supports the idea that California gall wasps are ecosystem engineers and have long lasting effects on the ecosystem through habitat modification. Not only do cynipid gall wasps influence the foliage-dwelling arthropod community on valley oak (Wetzel et al., 2014), they can potentially influence the arthropod community under valley oak trees at ground level. The addition of a

layer of galls above soil can introduce inhibitory compounds such as tannins, which can have effects beyond the original purpose of cynipid gall manipulation: to provide refuge and nutritive tissue to cynipid larvae.

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