5-5-2017

Friends, Enemies, or Hitchhikers? Exploring the Relationship Between Fungus, Butterfly, and Rest Plant at Fort Jackson, South Carolina

Miranda F. Hannah

Director of Thesis: Dr. Carol Boggs
Second Reader: Dr. Meredith Blackwell

Recommended Citation

Hannah, Miranda F., "Friends, Enemies, or Hitchhikers? Exploring the Relationship Between Fungus, Butterfly, and Rest Plant at Fort Jackson, South Carolina" (2017). *Senior Theses*. 162.
https://scholarcommons.sc.edu/senior_theses/162

Table of Contents

Thesis Summary 3

Abstract 5

Introduction 5

Materials and Methods 8

Results 12

Discussion 15

Acknowledgements 19

Supplemental Tables 20

References 27

Thesis Summary

My thesis investigated whether or not butterflies are carriers of fungi and if so, what species of fungi are present on the legs and abdomens of the butterflies. Butterflies were captured on flowers at McCrady Training Center, Fort Jackson, South Carolina, where the lab of Dr. Carol Boggs, which I became involved with during my sophomore year at the University of South Carolina, had a research grant to conduct a general survey and look for monarch butterflies. While other members of the lab were surveying for pollinators, I was capturing *Euptoieta claudia* (Lepidoptera: Nymphalidae), a species of butterfly that typically flies in the late summer and fall. The plants on which the butterflies had been resting or feeding before capture was noted. Upon capture, I walked the butterflies across agar plates so that their legs and abdomens touched the media. Agar is a nutritious solution that promoted the growth of whatever microbes were on the legs and abdomens of the butterflies. Once the plates had enough fungal growth, the fungi were isolated and purified before being identified using a compound microscope.

The most common fungi isolated from the butterflies were *Fusarium*, *Penicillium*, *Curvularia*, *Cladosporium*, and *Aspergillus*. These fungi have positive and negative implications for insects, plants, and even humans. Species of *Penicillium*, *Cladosporium*, *Aspergillus*, and *Fusarium* have been used in a number of medicines including antibiotics, antifungals, and medications that lower cholesterol. *Aspergillus* is used in production of soy sauce and the citric acid that eventually goes in soda products. *Penicillium* is a component in several popular cheeses including gorgonzola and brie.

In contrast, *Fusarium* produces toxins that have been known to damage other insects. Some *Aspergillus* species produce carcinogenic compounds and other species can cause infections in humans that are potentially fatal. *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium* are all fungal allergens to humans. Many of the species of fungal genera found in this study also produce plant diseases such as leaf spots.

This study also found that butterflies previously resting on *Passiflora incarnata* flowers had a larger number of individual fungal species present. This could be due to *Passiflora* having a more open nectar reserve than the other flowers examined in this study. The number of fungal species present per butterfly fit a Poisson distribution, meaning the number of fungal species present per butterfly appeared to be due to chance. These results could mean that butterflies are picking up fungal species from wherever they land and carrying those species for a time.

Future studies should take larger samples of butterfly microbes from a variety of species of butterfly rather than just the one species. Additionally, flower petals, leaves, and nectar reserves should be analyzed as well to look for correlations between flower elements and fungal species. Taking samples of where butterflies land other than on plants would also be an interesting study to see what fungi they pick up. These and other future studies could greatly increase the scientific knowledge of the roles butterflies play in the spread of fungi and other microbes to plants and humans. This information could be harnessed to use butterflies as transporters of beneficial fungi that could protect the plants the butterflies visit from pests as well as the butterflies themselves from predators. So much is still unknown on the exact role of fungi in the lives of butterflies. But this study opens many doors and raises many questions that could be investigated in future studies.

Abstract

This study was undertaken to investigate the relationships between butterflies as pollinators, host plants, and fungi. *Euptoieta claudia* (Lepidoptera: Nymphalidae) butterflies were captured on flowers at McCrady Training Center, Fort Jackson, South Carolina and the fungi isolated from the legs and abdomens of these butterflies was identified and analyzed. Butterflies were found to carry a variety of fungal species including *Penicillium*, *Cladosporium*, *Curvularia*, *Fusarium*, and *Aspergillus*. Butterflies carrying the highest quantity of different species of fungi were captured on *Passiflora incarnata* flowers, and the number of fungal species per butterfly seems to be due to chance. The most common species of fungi found on butterflies in this study have important medicinal and industrial applications. Also, butterflies possibly transfer the fungi from plant to plant and this could impact the spread of plant and pollinator diseases.

Introduction

The role of microbes in the lives of insects is a topic of increasing interest. Insects and fungi have evolved in the same habitats for many millions of years, so it is not surprising that they should interact in their close quarters. Emphasis in microbial-insect pollinator interactions is high, but based on my review of the literature information on butterflies and their microbial associates is less widely available than that of fungi and bee pollinators.

Fungi are known to parasitize Lepidoptera in the larval and pupal stages, and certain fungal species have been suggested as agents for biological control, but the relationship between butterflies as pollinators and the bacteria, yeasts, and other fungi that adhere to their surfaces from the flowers they pollinate is less well known.

As with bees, when a butterfly lands on a flower to feed, pollen from that flower is transferred to the butterfly. Transfer of this pollen to other flowers the butterfly visits promotes cross pollination. Along with the transfer of pollen, the butterfly could very likely also be transferring microbes from plant to plant. Hummingbirds have been shown to be vectors of *Fusarium moniliforme* spores in a possibly mutualistic relationship (Lara and Ornelas, 2003). The diseased plants flowered longer, providing more nectar for visiting hummingbird vectors to increase the chances of transfer of fungal spores to other plants (Lara and Ornelas, 2003). Of particular interest in South Carolina is the possibility that the Palamedes Swallowtail (*Papilo palamedes*), which uses plants in Lauraceae, especially red bay as food plants, could spread a deadly, recently introduced disease (Fraedrich et al. 2007), among these susceptible plants.

Other interactions this entails could be beneficial or detrimental to the plant or butterfly. A study found that a butterfly infected by a protozoan parasite flew on average 20% less well than healthy butterflies (O’Brien and Walton, 2012). This can greatly affect butterflies that must migrate such as the monarch butterfly (O’Brien and Walton, 2012). There are several other examples of diseases of butterflies related to bacteria and fungi, such as *Bacilus thuringiensis*

(Bt), a bacterium used as a natural pest control by many nurseries to kill worms or other pests that feed on milkweed and other plants (Monarch Butterfly Diseases).

Some positive implications of microbes associated with insects are known. Bees, another common pollinator, were used in successful experiments to distribute fungicides to plants in a safer and more economical way (Fellows, 2013). As the bees exited the hive, they were coated with a beneficial fungus that is just as successful as synthetic fungicide spraying (Fellows, 2013). This was shown to naturally increase crop quality and yield (Fellows, 2013). Because pesticides are not always effective and result in environmental and health hazards, butterflies may be another diverse pollinator that could be used to naturally control which fungi and pests are present. This could prevent many known butterfly killers such as chalcid wasps from eating butterflies during their chrysalis stage by providing that natural pesticide when the maternal butterfly deposits her eggs (Monarch Butterfly Diseases).

In this study, I investigated the distribution patterns of *Euptoieta claudia, Papilo palamedes*, and *Papilo troiles* in McCrady Training Center, Fort Jackson, South Carolina. I hypothesized that the microbiota found on the abdomens and legs of the butterflies would be correlated with the food and, perhaps, rest plants that species of butterflies used before capture. Because of the timing of the collection to correlate with the school term, most of the butterflies collected were *Euptoieta claudia*. The study, therefore, developed into one that emphasized the single species.

Materials and Methods

Collection of Butterflies.

Butterflies of the species *Euptoieta claudia* were collected from Sites 1 and 4 of McCrady Training Center at Fort Jackson, South Carolina (Fig. 1). They were caught with a butterfly net and carefully gripped by the wings using forceps; then they were made to walk across agar plates so that their legs and some of their abdomen touched the agar. The type of plant the butterflies were resting on at capture was noted. The butterflies were then released and the agar plates were sealed with Parafilm and incubated for several days so that any yeast, other fungus, or bacterium transferred from the butterflies could grow.

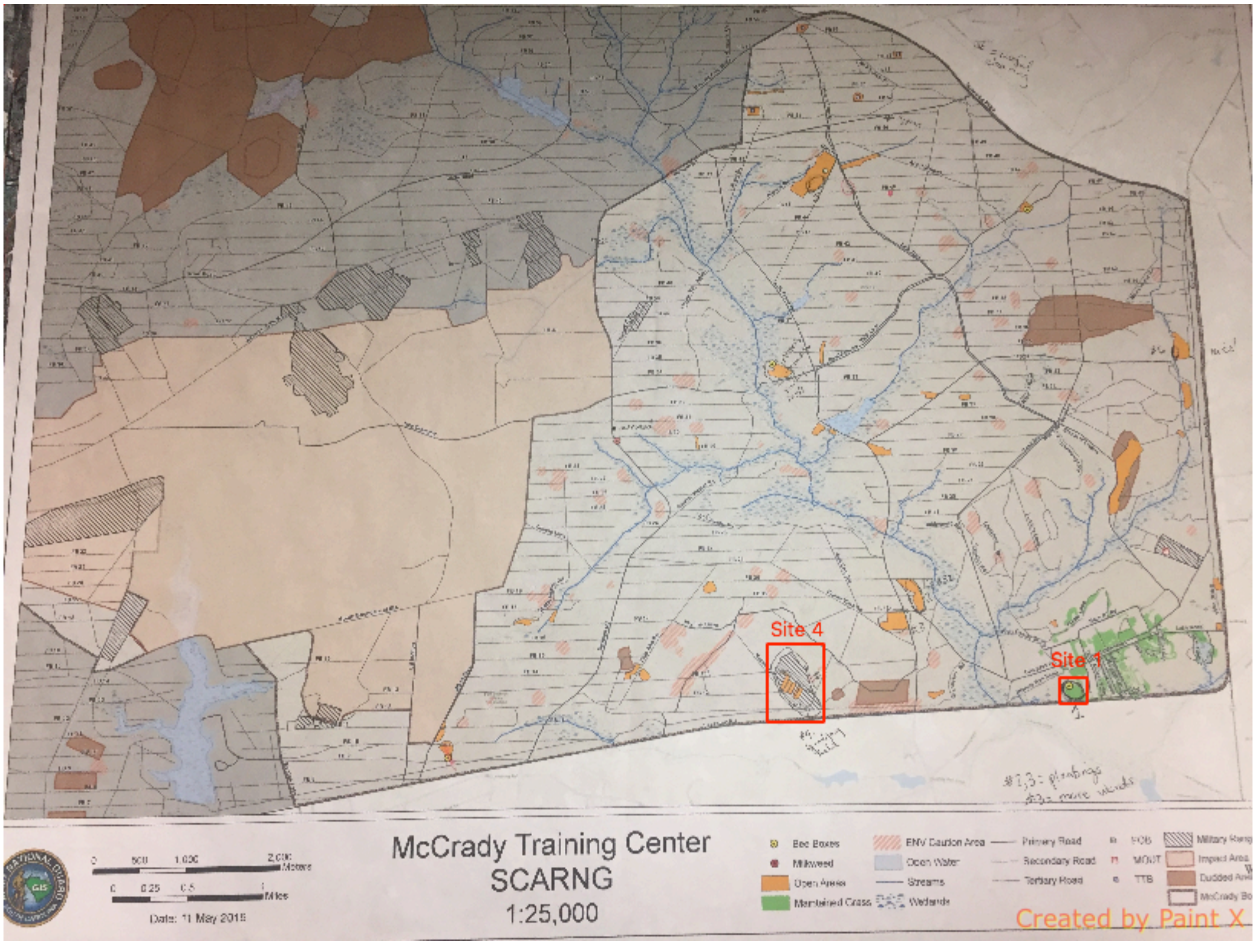

Figure 1. Map of McCrady Training Center, SC Army National Guard, Ft. Jackson, SC, 5411 Lessburg Rd, Eastover, SC 29044. Sites 1 and 4 where the study was done are marked on the map.

Preparation of culture media.

Three types of agar (½ corn meal agar, ½ malt agar, and YM agar, see below) were used in this study based on availability of materials. They were usually made in batches of 1 liter at a time, using 500 mL and 1 L volume autoclavable plastic flasks that were filled up halfway with medium, covered with tin foil, and placed in an autoclavable bin so the water would not overflow in the autoclave for 15 minutes at 121C. Agar was poured into 9 centimeter petri dishes so that each dish was filled halfway and let cool for one day to detect potential contamination before being stored in a laboratory refrigerator.

Agar media:

Half strength corn meal agar: 4.25 grams of corn meal agar and 4-5 grams of bacteriological agar in 500 milliliters of distilled water.

Half strength malt agar: 2.5 grams of malt agar and 5.0 grams of bacteriological agar in 500 milliliters of distilled water.

YM agar: 10 grams of yeast malt extract, bacteriological agar in 500 milliliters of distilled water.

Purification of cultures.

Once plates had sufficient growth, the fungal growth was purified by transfer to a new agar plate using sterile isolation methods. First the isolation probe was soaked in 95% ethanol before being held in the flame of an alcohol lamp for roughly five seconds. The isolation probe was once again dipped in the alcohol to cool off the metal. Then the probe was used to take a small sample of the microbiota on the agar plate which was transferred to a new, clean agar plate. The isolation probe was sterilized between each use until all microbiota were transferred to new plates. Plates with multiple species on them were transferred in such a way that each species got its own new plate and so that each species was isolated in pure culture. Plates were allowed to grow for 2-3 days before being isolated again using sterile isolation techniques.

Cultures were purified three times, roughly three days apart. Isolation techniques were the same as in the above paragraph. A microscope was used to determine areas of clean fungal growth for transfer on the agar plates so that no contamination would occur.

Cultures were transferred one last time to ½ malt or ½ corn meal agar plates. ½ strength media was used because the lower quantity of nutrients make the fungal growth more likely to form conidia. Three strains were placed equidistant to each other on the plate and labeled to match the plate they came from.

Identification of cultures.

Roughly 2-3 days after final transfer, the fungal growth was analyzed. Fungal growth was sorted according to phenotype and identified using a compound microscope at X100 to look at how the conidia developed on the mycelium.

A microscopic mount was made when necessary for proper identification. Sterile methods were used to take a small sample of the growth with as little agar as possible. The sample was placed on a slide and viewed under a microscope. Fungal growth was identified in this way by looking at where the conidial color, shape, and development on the hyphae.

Identification manuals used to identify the fungal growth, either by using the microscope to look at the agar plate or the microscope mount, included Barnett and Hunter (1990), Barron (1968), Smith (1990), and Wang and Zabel (1990).

Statistical Analyses.

Excel was used to record and organize data taken in the field, and SYSTAT13 software was used for statistical analyses. Fisher's Least-Significant-Difference Test was used to compare similarity between plants. Number of fungal species per butterfly data were tested for Poisson distribution.

Results

At least one fungal species was found on each butterfly captured. Table 1 below shows the number of *E. claudia* butterflies from which each fungus found was isolated.

Fungus species	Number of butterflies from which fungus was isolated
Cladosporium	9
Fusarium	8
Curvularia	6
Penicillium	5
Aspergillus	4
Papularia	2
Alternaria	1
Stemphyllium	1
Aureobasidium	1

Table 1. Number of *E. claudia* butterflies from which each fungal species found was isolated.

The number of fungal species isolated from butterflies of each plant was analyzed using Fisher's Least Significant Difference Test. Table 2 below shows the p-values obtained from this analysis. This comparison of plant species richness is shown in Figure 2.

Fisher's Least-Significant Difference Test		
Plant (i)	Plant (j)	p-value
Passiflora incarnata	*Erigeron strigosus*	0.048
Passiflora incarnata	*Verbena brasiliensis*	0.044
Passiflora incarnata	*Helenium amarum*	0.028
Passiflora incarnata	*Solidago nemoralis*	0.825
Erigeron strigosus	*Verbena brasiliensis*	0.834
Erigeron strigosus	*Helenium amarum*	0.755
Erigeron strigosus	*Solidago nemoralis*	0.203
Verbena brasiliensis	*Helenium amarum*	0.578
Verbena brasiliensis	*Solidago nemoralis*	0.225
Helenium amarum	*Solidago nemoralis*	0.143

Table 2. Comparison of number of fungal species isolated from butterflies previously resting on each plant using Fisher's Least Significant Difference Test.

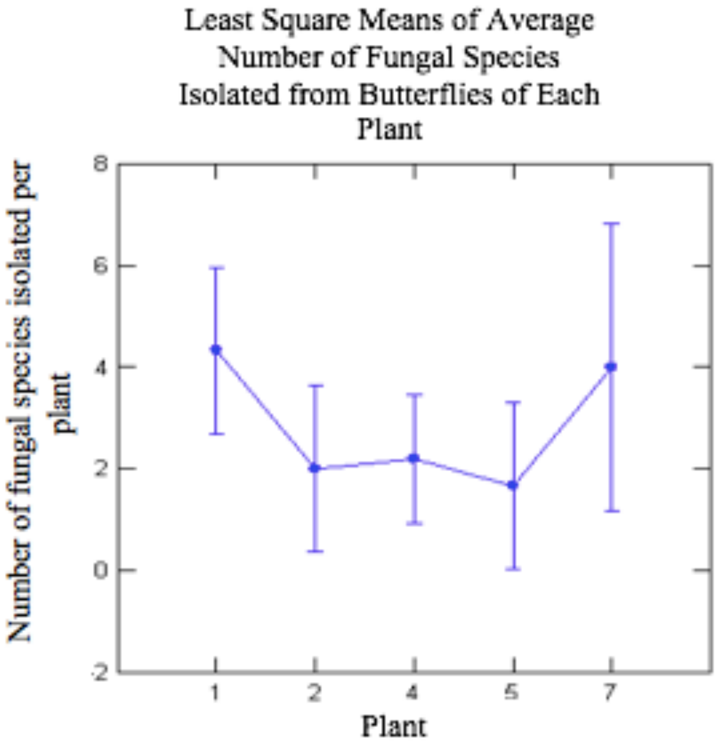

Figure 2. Least square means of average number of fungal species isolated from E. claudia butterflies of each plant. Plants are assigned to the following numbers on the graph: *Passiflora incarnata* (1), *Erigeron strigosus* (2), *Verbena brasiliensis* (4), *Helenium amarum* (5), and *Solidago nemoralis* (7).

The number of fungal species isolated from each butterfly was analyzed as well to determine if it fits a Poisson distribution. Figure 3 is a scatterplot measuring number of fungal species present per butterfly against a Poisson quantile. Table 3 shows the Poisson numerical analysis of this data for number of fungal species present.

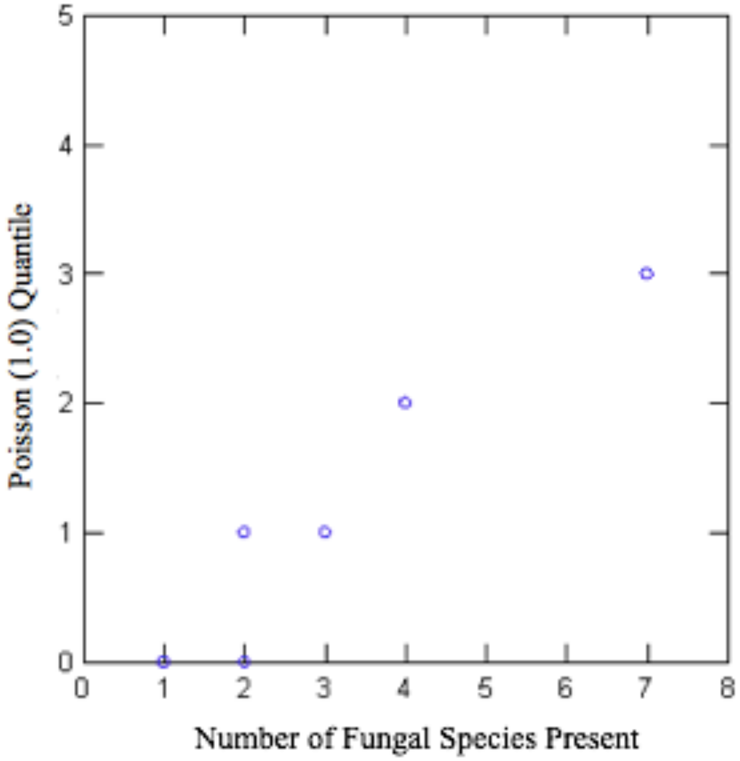

Figure 3. Number of fungal species present per *E. claudia* butterfly in a Poisson quantile.

N of cases	15
Minimum	1
Maximum	7
Arithmetic mean	2.667
Standard deviation	1.543
Variance	2.381

Table 3. Numerical analysis of number of fungal species present per butterfly using the Poisson analysis.

Supplemental Tables 4-9 (see below) at the end of this paper list the species of fungi isolated from plants in the Southeastern United States according to U. S. National Fungus Collections Fungus-Host Database. The fungal species found on the plants in this study have an asterisk (*) next to them.

Discussion

This study sought to investigate whether or not microbes are found on the abdomens and legs of butterflies and if the microbes found could be correlated with the food and perhaps rest plants the butterflies were using at capture. All of the samples taken exhibited the growth of at least one fungus, which indicates that butterflies are transporters of fungi. It is possible that pollinators spread the fungi from plant to plant. The most common fungi found were *Cladosporium*, *Fusarium*, *Curvularia*, *Pencillium*, and *Aspergillus*.

Tables 4-9 list the species of fungi isolated from plants in the Southeastern United States. An asterisk (*) was placed next to the species in each table that was also found in the fungal samples. *Curvularia* was not present on any of the plants searched in the database. A possible reason for this is that the butterflies were visiting flowers other than those on their regular host comprehensive list, meaning *Curvularia* might not be present simply because the database is not up to date. Table 10 lists the plants in the Southeastern United States reported to host *Curvularia* according to the U. S. National Fungus Collections Fungus-Host Database (Farr and Rossman, 2017).

No correlations between species of fungi and rest plants were found, possibly because of the limited sample size taken. However, *Passiflora incarnata* was found to be significantly different from three other plants analyzed in number of fungal species per plant. This could be because *Passiflora incarnata* has more open and accessible nectaries that are more easily contaminated with fungi and other microbes. But the host family *Passifloraceae* does not have as many reported fungal species in the U. S. National Fungus Collections Fungus-Host Database for the Southeastern United States (Table 7). This could be because the database is not comprehensive or not often updated. Another possibility is that the butterflies are picking up fungi while scratching leaves of the *Passiflora incarnata* plant to deposit eggs, as *Passiflora incarnata* is a host plant of *E. claudia*.

The number of fungal species per butterfly was found to fit a Poisson distribution (the scatterplot shows a straight line) which indicates that the number of species present per butterfly is determined by chance. Another way to tell if the data fit a Poisson distribution is if the mean is equal to the variance, and in this case the mean (2.667) and variance (2.381) are very close to each other. This chance determination of number of fungal species per butterfly could mean that the butterflies are picking up fungal species from wherever they land and carrying those species for a time.

Fungi have been linked to important benefits for insects. Rather than being pathogens or parasites, ascomycete yeasts provide important vitamins, enzymes, among other metabolites (Vega and Kaya 2012). Butterflies could be forcibly exposed to certain beneficial fungi. If butterflies can drop fungi when they deposit their eggs, having butterflies coated in beneficial fungi that repel pests can prevent these insects such as the chalcid wasp from destroying the butterfly eggs. Exposing butterflies to these beneficial fungi could lead to more information on the mechanics to prevent butterfly diseases. This could have a beneficial impact on species conservation of butterflies or other insects.

The most common species of the fungi found on these butterflies have important medical or industrial applications. At the beginning of the 21st century, fungi were involved in the industrial processing of 10-20 of the most profitable products in human medicine including anti-cholesterol medicines, penicillin, and the immuno-suppressant cyclosporin A (Drugs from Fungi 2004). *Penicillium chrysogenum* is used to make the antibiotic penicillin, and *Penicillium griseofulvin* is used to make an antifungal agent called griseofulvin (Drugs from Fungi 2004). *Aspergillus fumigatus* produces gliotoxins which have antibiotic activity, and *Aspergillus terreus* produces a secondary metabolite called lovastatin which lowers cholesterol (Drugs from Fungi 2004). On species of *Cladosporium* is able to transform some steroidal compounds into progesterone (Mold Awareness). Also, *Fusarium* has potential application as an anabolic steroid and growth stimulant (EM Lab P&K). Yeasts play a role in fermentation in brewing, wine making, and bread making (Seidl). *Aspergillus* is used in the production of some of the more common fermented foods of Asia such as soy sauce and soy bean paste (Seidl). Citric acid in soft drinks is produced in large scale fermentation vats using *Aspergillus* (Seidl). *Penicillium* can be found in some cheeses such as gorgonzola and blue cheeses (Seidl). Butterflies could be used to control pests via fungicide transportation such as with the experiment done with bees that successfully used them as a vector to control pests (Fellows 2013).

While the fungi found on these butterflies do have many beneficial medicinal and industrial applications, they also have some negative implications to consider. *Fusarium* produces a toxic compound called beauvericin which, when examined, damaged fall armyworm cell lines and the ultrastructure of mosquito larvae (Vega and Kaya 2012). Certain species of *Aspergillus* and *Penicillium* are entomopathogens as well (Vega and Kaya 2012). Fungi can also cause infections in humans that range from rashes to systemic mycoses that can be potentially fatal (Horner, Helbling, Salvaggio & Lehrer, 1995). Some fungi such as *Aspergillus falvus* and *Aspergillus parasiticus* also produce toxins that are carcinogenic (Horner, Helbling, Salvaggio & Lehrer, 1995). Fungal spores are now recognized as causes of some respiratory allergies, including *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., and *Penicillium* spp. (Horner, Helbling, Salvaggio & Lehrer, 1995). Besides picking up these allergens from contact with insects, humans can pick up these allergens from the air or by touching surfaces or plants.

Many species of the fungal genera that were found in this study can also cause plant diseases such as leaf spots. Most plants are susceptible to leaf spot diseases. Because it is possible that butterflies as pollinators can spread fungi from plant to plant, this would indicate that plant diseases caused by fungi could also be spread by butterflies. While wounding is not required to spread fungi that cause leaf spots, wounding can trigger increased disease expression and may result in faster development of leaf spots (Blodgett & Swart, 2002). This was seen in the study done by Blodgett and Swart (2002) which investigated the infection of *Amaranthus hybridus* by *Alternaria tenuissima*. Alternaria, a fungus found in the current study, has been shown to cause leaf spots in other economically important plants as well, such as potato, tomato, and onion leaves (Blodgett and Swart, 2002). Butterflies could potentially be spreading fungi from plant to plant and may cause an increased rate of development of leaf spots when maternal butterflies scratch leaves to deposit their eggs.

Future studies should take larger samples of butterfly walks with a variety of species of butterflies rather than just one species. Additionally, flower petals, leaves, and nectaries should be analyzed as well to look for correlations between flower elements and fungal species. Taking samples of other surfaces where butterflies land would also be an interesting study to see what fungi they pick up. Studies could also analyze whether there are cross-generational effects of carrying fungi, such as whether maternal butterflies pass on fungi to the eggs they deposit or whether juvenile butterflies carry similar fungi to their parental butterflies. Because the butterflies on *Passiflora incarnata* carried more species of fungi and *Passiflora incarnata* is a host plant for *Euptoieta claudia*, differences in number and fungal species between male and female butterflies could also be analyzed as well as time spent on each plant. In the future, fungi from leaf spots can be identified by DNA.

Acknowledgements

I would like to thank Dr. Carol Boggs for being my thesis director and offering assistance, especially with statistics, in this project. I would like to thank Dr. Meredith Blackwell for all of the hours spent helping me with this project in the lab and for all of the helpful edits. I would also like to thank Nicole Kish for her initial assistance in data collection at McCrady Training Center and for answering the periodic questions I would send her. Finally, I would like to thank the South Carolina Honors College and the University of South Carolina for this opportunity.

Supplemental Tables

Flower Name	**Fungus reported on flower**	**Area reported**
Cirsium altissimum	Acrospermum follicola	Georgia
Cirsium altissimum	Botryosphaeria dothidea	Georgia
Cirsium altissimum	Diaporthe arctii	Georgia
Cirsium altissimum	Metasphaeria	Georgia
Cirsium altissimum	Ophiobolus anguillides	Georgia
Cirsium altissimum	Puccinia altissimorum	North Carolina
Cirsium altissimum	Puccinia cirsii	Texas
Cirsium arvense	Septoria cirsii	Mississippi
Cirsium horridulum	Albugo tragopogonis	Florida
Cirsium horridulum	Erysiphe cichoracearum	Florida
Cirsium horridulum	Puccinia altissimorum	Louisianna
Cirsium horridulum	Puccinia cirsii	Florida
Cirsium horridulum	Uromyces junci	Florida
Cirsium lanceolatum	Puccinia cirsii	Mississippi
Cirsium muticum	Heptameria obesa	South Carolina
Cirsium muticum	Leptosphaeria mesoedema	South Carolina
Cirsium undulatum	Puccinia inclusa	Texas
Cirsium vulgare	Piccinia cnici	Mississippi

Table 4. Fungi found on plants of the host genus *Cirsium* in the Southeastern United States according to the U. S. National Fungus Collections Fungus-Host Database (Farr and Rossman 2017).

Flower Name	**Fungus reported on flower**	**Area reported**
Erigeron strigosus	Basidiophora entospora	North Carolina
Erigeron strigosus	Basidiophora	Alabama
Erigeron strigosus	Cercospora cana	Texas
Erigeron strigosus	Cercosporella virgaureae	North Carolina, Texas
Erigeron strigosus	Erysiphe cichoracearum	North Carolina
Erigeron strigosus	Leptothyrium punctiforme	West Virginia
Erigeron strigosus	Puccinia cyperi	Texas
Erigeron strigosus	Puccinia extensicola	Mississippi, Texas
Erigeron strigosus	Septoria erigerontis	North Carolina, Texas

Table 5. Fungi found on plants of host name *Erigeron strigosus* in the Southeastern United States according to the U. S. National Fungus Collections Fungus-Host Database (Farr and Rossman 2017).

Flower Name	**Fungus reported on flower**	**Area reported**
Helenium autumnale	Entyloma compositarum	Texas
Helenium autumnale	Phomopsis	Mississippi
Helenium autumnale	Phymatotrichum omnivorum	Texas

Helenium autumnale	Puccinia extensicola	Texas
Helenium commune	Hypoderma commune	Georgia
Helenium microcephalum	Cercospora helenii	Texas, United States
Helenium tenuifolium	Cercospora helenii	Alabama, United States
Helenium tenuifolium	Erysiphe cichoracearum	Texas
Helenium tenuifolium	Metasphaeria sanguinea	Alabma
Helenium tenuifolium	Phymatotrichum omnivorum	Texas
Helenium tenuifolium	Pleospora scrophulariae	Georgia
Helenium tenuifolium	Synchytrium macrosporum	Texas

Table 6. Fungi found on plants of the host genus Helenium in the Southeastern United States according to the U. S. National Fungus Collections Fungus-Host Database (Farr and Rossman 2017).

Flower Name	**Fungus reported on flower**	**Area reported**
Passiflora	Asterina perconferta	United States
Passiflora	Cercospora fuscovirens	Texas
Passiflora	Cercospora passiflorae	Florida
Passiflora	Cercospora regalis	Texas
Passiflora	Cercospora truncatella	Alabama, North Carolina, Texas
Passiflora	Colletotrichum boninense	Florida
Passiflora	Colletotrichum capsici	Florida
Passiflora	Colletotrichum gloeosporioides	Florida
Passiflora	Corynespora cassiicola	Florida
Passiflora	Gloeosporium fructigenum	Louisiana
Passiflora	Glomerella	Florida
Passiflora	Phymatotrichum omnivorum	Texas
Passiflora	Pythium splendens	Florida
Passiflora	Rhizoctonia solani	Florida
Passiflora	Sclerotium rolfsii	Florida
Passiflora	Septoria fructigena	South Carolina
Passiflora coriacea	Colletotrichum capsici	Florida
Passiflora coriacea	Myrothecium roridum	Florida
Passiflora edulis	Alternaria passiflorae*	Florida
Passiflora edulis	Alternaria tropica*	Florida
Passiflora edulis	Botryodiplodia	Florida
Passiflora edulis	Colletotrichum acutatum	Florida
Passiflora edulis	Colletotrichum gloeosporioides	Florida
Passiflora edulis	Fusarium oxysporum*	North America
Passiflora edulis	Fusarium*	Florida
Passiflora edulis	Phyllosticta	Florida
Passiflora edulis	Septoria passiflorae	Florida
Passiflora incarnata	Cercospora apii	Alabama

Passiflora incarnata	Cercospora biformis	Arkansas
Passiflora incarnata	Cercospora fuscovirens	North Carolina
Passiflora incarnata	Cercospora truncatella	Alabama, North Carolina
Passiflora incarnata	Leptosphaeria subconica	Georgia
Passiflora lutea	Cercospora fuscovirens	Missouri, South Carolina
Passiflora lutea	Synchtrium	Louisianna

Table 7. Fungi found on plants of the host family *Passifloraceae* in the Southeastern United States according to the U. S. National Fungus Collections Fungus-Host Database (Farr and Rossman 2017). Asterisk (*) indicates fungal genus associated with *Euptoieta claudia* in this study.

Flower Name	**Fungus reported on flower**	**Area reported**
Solidago nemoralis	Coleosporium solidaginis	Georgia
Solidago nemoralis	Diaporthe linearis	Georgia
Solidago nemoralis	Leptosphaeria comatella	Georgia
Solidago nemoralis	Leptosphaeria	Georgia

Table 8. Fungi found on plants of the host *Solidago nemoralis* in the Southeastern United States according to the U. S. National Fungus Collections Fungus-Host Database (Farr and Rossman 2017).

Flower Name	**Fungus reported on flower**	**Area reported**
Alovsia lycoides	Propodium lippiae	Texas
Avicennia germinans	Acremonium	Texas
Avicennia germinans	Alternaria*	Texas
Avicennia germinans	Aspergillus flavus*	Texas
Avicennia germinans	Aspergillus niger*	Texas
Avicennia germinans	Aspergillus terreus*	Texas
Avicennia germinans	Camarosporium roumeguerii	Texas
Avicennia germinans	Cladosporium cladosporioides*	Texas
Avicennia germinans	Cladosporium oxysporum*	Texas
Avicennia germinans	Dendryphiella arenaria	Texas
Avicennia germinans	Drechslera	Texas
Avicennia germinans	Epicoccum nigrum	Texas
Avicennia germinans	Fusarium*	Florida, Texas
Avicennia germinans	Gnomonia longirostris	Texas
Avicennia germinans	Hydronectria Tethys	Florida
Avicennia germinans	Leptosphaeria avicenniae	Florida
Avicennia germinans	Leptosphaeria Halima	Texas
Avicennia germinans	Mycosphaerella pneumatophorae	Florida
Avicennia germinans	Mycovellosiella	Florida
Avicennia germinans	Nigrospora sphaerica	Texas

Avicennia germinans	Periconia byssoides	Texas
Avicennia germinans	Pestalotia macrotricha	Texas
Avicennia germinans	Phoma	Texas
Avicennia germinans	Phyllosticta hibiscina	Florida
Avicennia germinans	Pleospora pelagica	Texas
Avicennia germinans	Pythium	Florida
Avicennia germinans	Rhabdospora avicenniae	Florida
Avicennia germinans	Stemphylium*	Texas
Avicennia germinans	Trichoderma viride	Texas
Avicennia germinans	Ulocladium botrytis	Texas
Duranta repens	Cercospora durantae	Florida
Duranta repens	Phyllachora fusicarpa	Florida
Duranta repens	Rhizoctonia	Florida
Duranta repens	Sclerotium rolfsii	Florida
Lantana	Acanthostigma floridense	Florida
Lantana	Alternaria*	Florida, Texas
Lantana	Cercospora lantanae	Florida
Lantana	Collectotrichum	Florida
Lantana	Diatrypella lantanae	Florida
Lantana	Meliola cookeana	Florida
Lantana	Phyllosticta	Florida
Lantana	Physalospora obtuse	Alabama
Lantana	Pythium	Florida
Lantana	Ramularia	Florida
Lantana	Rhizoctonia solani	Florida
Lantana camara	Cercospora lantanae	Florida
Lantana camara	Corynespora cassiicola	Florida
Lantana camara	Diatrypella lantanae	Florida
Lantana camara	Meliola cookeana	Florida
Lantana camara	Phytophthora parasitica	Louisiana
Lantana camara	Puccinia lantanae	Florida
Lantana camara	Rhizoctonia solani	Florida
Lantana involucrata	Meliola cookeana	Florida
Lantana involucrata	Puccinia lantanae	Florida
Lantana involucrata	Sclerotium rolfsii	Florida
Lantana macropoda	Puccinia versicolor	Texas
Lantana montevidensis	Cercospora lantanae	Florida
Lantana montevidensis	Fusarium oxysporum*	Florida
Lantana montevidensis	Meliola cookeana	Florida
Lantana montevidensis	Puccinia lantanae	Florida
Lippia	Cercospora lippiae	Texas
Lippia	Meliola lippiae	Florida

Lippia	Phymatotrichum omnivorum	Texas
Lippia	Sphaceloma lippiae	Florida
Lippia lancelata	Cercospora lippiae	Mississippi
Lippia lingustrina	Cylindrosporium lippiae	Texas
Lippia nodiflora	Cercospora lippiae	Florida, Louisiana
Lippia nodiflora	Meliola ambigua	Florida
Lippia nodiflora	Meliola lippiae	Florida
Lippia nodiflora	Oidium	Florida
Lippia nodiflora	Sphaceloma lippiae	Florida
Lippia stoechadifolia	Meliola lippiae	Florida
Lippia stoechadifolia	Puccinia lantanae	Florida
Phyla incisa	Synchytrium macrosporum	Texas
Phyla nodiflora	Meliola lippiae	Florida
Phyla nodiflora	Phomopsis	Mississippi
Phyrma leptostachya	Septoria leptostachya	Mississippi
Stachytarpheta	Cercospora stachytarphetae	Florida
Stachytarpheta	Puccinia urbaniana	Florida
Verbena	Cercospora papillosa	Alabama
Verbena	Cercospora truncatella	Alabama
Verbena	Cercospora verbenicola	Alabama, Louisiana, Texas
Verbena	Erysiphe cichoracearum	Georgia
Verbena	Fusarium oxysporum*	Florida
Verbena	Septoria verbenae	Mississippi, Texas
Verbena bipinnatifida	Cercospora verbenicola	Texas
Verbena bipinnatifida	Phyllosticta verbenicola	Texas
Verbena bipinnatifida	Phymatotrichum omnivorum	Texas
Verbena bipinnatifida	Septoria verbenae	Texas
Verbena carnea	Cercospora septatissima	Mississippi
Verbena carnea	Sirosporium septatissima	Mississippi
Verbena caroliniana	Cercospora septatissima	Mississippi
Verbena caroliniana	Sirosporium septatissima	Mississipii
Verbena hastata	Cercospora	Mississippi
Verbena hastata	Collectotrichum	Mississippi
Verbena hastata	Phomopsis	Mississippi
Verbena hastata	Septoria verbenae	Mississippi, Texas
Verbena horrida	Phymatotrichum omnivorum	Texas
Verbena hybrida	Alternaria*	Florida
Verbena hybrida	Cercospora	Florida
Verbena hydrida	Erysiphe cichoracearum	Florida, North Carolina
Verbena hybrida	Phymatotrichum omnivorum	Texas
Verbena hybrida	Phytophthora cryptogea	North Carolina
Verbena hybrida	Phytophthora nicotianae	North Carolina
Verbena hybrida	Phytophthora	Florida
Verbena hybrida	Phytophthora tropicalis	North Carolina
Verbena hybrida	Pythium	Florida

Verbena hybrida	Rhizoctonia solani	Florida
Verbena stricta	Erysiphe cichoracearum	Mississippi
Verbena stricta	Phyllosticta texensis	Texas
Verbena stricta	Septoria verbenae	Mississippi, Texas
Verbena urticifolia	Erysiphe cichoracearum	North Carolina
Verbena urticifolia	Septoria verbenae	Mississippi, Texas
Verbena xutha	Cercospora verbenicola	Louisiana

Table 9. Fungi found on plants of the host family *Verbenaceae* in the Southeastern United States according to the U.S. National Fungus Collections Fungus-Host Database (Farr and Rossman 2017). Asterisk (*) indicates fungal genus associated with *Euptoieta claudia* in this study.

Plant Name	**Fungus reported on plant**	**Area reported**
Aeschynanthus pulcher	Curvularia	Florida
Agrostis	Curvularia	North Carolina
Alocasia	Curvularia	Florida
Araucaria heterophylla	Curvularia	Florida
Aristida	Curvularia	Florida
Axonopus affinis	Curvularia	Georgia, North Carolina
Baccharis halimifolia	Curvularia	Florida
Camellia	Curvularia	Southeastern states
Cassia obtusifolia	Curvularia	Florida
Cassia tora	Curvularia	Mississippi
Cenchrus	Curvularia	Florida
Chamaedorea elegans	Curvularia	Florida
Chloris	Curvularia	Florida
Cortaderia selloana	Curvularia	Florida
Cynodon dactylon	Curvularia	North Carolina
Dactylis glomerata	Curvularia	Alabama
Datura metel	Curvularia	Florida
Davallia trichomanoides	Curvularia	Florida
Dianthus armeria	Curvularia	Maryland
Digitaria	Curvularia	Florida
Dimorphotheca	Curvularia	Georgia
Dracaena	Curvularia	Florida
Eichhornia crassipes	Curvularia	Florida
Eremochloa ophiuroides	Curvularia	Louisiana, North Carolina
Erianthus	Curvularia	Florida
Eugenia	Curvularia	Florida
Euphorbia maculata	Curvularia	Mississippi
Festuca elatior	Curvularia	North Carolina
Ficus benjamina	Curvularia	Florida
Fittonia verschaffeltii	Curvularia	Florida
Glycine max	Curvularia	Mississippi
Gossypium barbadense	Curvularia	Florida
Gossypium hirsutum	Curvularia	Florida, Mississippi

Heliconia	Curvularia	Florida
Hemigraphis alternata	Curvularia	Florida
Hibiscus rosa-sinensis	Curvularia	Florida
Hibiscus tiliaceus	Curvularia	Florida
Hippeastrum	Curvularia	Florida
Howeia forsteriana	Curvularia	Florida
Ipomoea purpurea	Curvularia	Mississippi
Jacquemontia tamnifolia	Curvularia	Mississippi
Juncus roemerianus	Curvularia	Florida
Lolium multiflorum	Curvularia	North Carolina
Lolium perenne	Curvularia	United States
Mangifera indica	Curvularia	Florida
Medicago sativa	Curvularia	Alabama
Oryza sativa	Curvularia	Florida
Pachira aquatic	Curvularia	Florida
Panicum	Curvularia	Maryland
Paspalum notatum	Curvularia	Florida
Paspalum	Curvularia	Florida
Pennisetum glaucum	Curvularia	Georgia
Phalaris arundinacea	Curvularia	Alabama
Philodendron scandens	Curvularia	Florida
Phleum pratense	Curvularia	Missouri
Physalis	Curvularia	Florida
Poa pratensis	Curvularia	North Carolina
Polianthes tuberosa	Curvularia	Florida
Saccharum officinarum	Curvularia	Louisiana
Setcreasea pallida	Curvularia	Florida
Sida spinose	Curvularia	Mississippi
Sorghum bicolor	Curvularia	Georgia
Sorghum vulgare	Curvularia	Georgia, Maryland
Spartina alterniflora	Curvularia	North Carolina
Stenotaphrum secundatum	Curvularia	Florida, North Carolina
Strelitzia reginae	Curvularia	Florida
Trifolium repens	Curvularia	South Carolina
Verbesina	Curvularia	Florida
Xanthium strumarium	Curvularia	Mississippi
Xyris ambigua	Curvularia	Florida
Zea mays	Curvularia	Florida, Mississippi
Zoysia japonica	Curvularia	North Carolina
Zoysia	Curvularia	North Carolina

Table 10. Plants of the Southeastern United States reported to host *Curvularia* according to the U. S. National Fungus Collections Fungus-Host Database (Farr and Rossman 2017).

References

Barnett, H. L. & Hunter B. B. 1998. Illustrated Genera of Imperfect Fungi. St. Paul, MN: APS Press.

Barron, G. L. 1968. The Genera of Hyphomycetes from Soil. Baltimore, MD: The Williams & Wilkins Company.

Blodgett, J. T. & Swart, W. J. 2002. Infection, colonization, and disease of *Amaranthus hybridus* leaves by the *Alternaria tenuissima* group. Plant Disease *86*: 1199-1205.

Cladosporium, a Common Mold Spore. *Mold Awareness*. Available from http://mold-awareness.org/cladosporium-common-mold-spore/

Drugs From Fungi. 2004. University of Sydney. Available from http://bugs.bio.usyd.edu.au/learning/resources/Mycology/UsesOf_Fungi/industrialProduction/fungalDrugs.shtml

Farr, D. F. & Rossman, A. Y. Fungal Databases, U. S. National Fungus Collections, ARS, USDA. Retrieved April 15, 2017, from https://nt.ars-grin.gov/fungaldatabases/

Fellows, K. Busy bees are controlling crop diseases and pests [Internet]. Pollination Canada, 2013 Feb [cited 2017 Mar 18]. Available from http://pollinator.org/nappc/PDFs/BusyBeesareControllingCropDiseasesandPests.pdf

Fraedrich, S. W., Harring, T. C., Rabaglis, R. J. 2007. Laurel Wilt: A new and devastating disease of redbay caused by a fungal symbiont of the exotic redbay Amrbosia beetle. Newsletter of the Michigan Entomological Society, 52: 15-16.

Horner, W. E., Helbling, A., Salvaggio, J. E., & Lehrer, S. B. 1995. Fungal Allergens. *Clinical Microbiology Reviews*. 161-179.

Lara, C. & Ornelas, J. F. 2003. Hummingbirds as vectors of fungal spores in *Moussonia deppeana* (Genseriaceae): Taking advantage of a mutualism? *American Journal of Botany*. 90(2):262-269.

Malloch, D. Moulds: Isolation, cultivation, identification. New Brunswick Museum. Available from http://website.nbm-mnb.ca/mycologywebpages/Moulds/MouldsDiscussion.html

Monarch Butterfly Diseases [Internet]. Butterfly fun facts [cited 2017 Mar 18]. Available from http://www.butterfly-fun-facts.com/disease-oe/monarch-butterfly-diseases/

O'Brien, M. & Walton, M. Butterflies and bats reveal clues about spread of infectious disease [Internet]. National Science Foundation; 2012 June [cited 2017 March 18]. Available from https://www.nsf.gov/news/special_reports/science_nation/butterfliesbats.jsp

Seidl, M. 2006. Industrial Uses of Fungi. The Environmental Reporter *4*(9). Available from https://www.emlab.com/s/sampling/env-report-09-2006.html

Smith, E. G. 1990. Sampling and Identifying Allergenic Pollens and Molds. San Antonio, TX: Blewstone Press.

Vega, F. E. & Blackwell, M. 2005. Insect-fungal associations: Ecology and evolution. New York, NY: Oxford University Press.

Vega, F. E. & Kaya, H. K. 2012. Insect pathology. San Diego, CA: Elsevier.

Wang, C. J. K. & Zabel, R. A. 1990. Identification Manual for Fungi from Utility Poles in the Eastern United States. American Type Culture Collection.