Quantifying the Effect of Pathways on the Distribution of Cynipid Galls in
Florida Pine Flatwood

By

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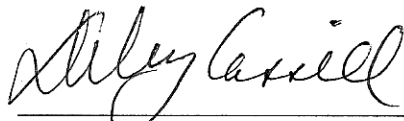
CERTIFICATE OF APPROVAL

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Honors Thesis

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This is to certify that the Honors Thesis of

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has been approved by the Examining Committee on April 17, 2017
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Table of Contents

Background

Plant-insect interactions are examples of coevolutionary processes found in symbiotic parasitism, mutualism, and commensalism. Aphids (superfamily *Aphidoidea*) parasitize a variety of leafy plants from which they draw sugar-rich sap, but usually do not kill or seriously hinder their host. Most *Pseudomyrmex* ant species benefit their *Acacia hindsii* host tree in a mutualistic relationship by removing pests, competing plants, and pathogens (González-Teuber *et al.*, 2014) while being fed by the tree's extrafloral nectaries. Flowers of the plant genus *Aristolochia* have a commensal relationship with the flies that they temporarily imprison before releasing, covered in pollen, without any nectar provisions but unharmed.

In each symbiotic relationship, at least one of the participants evolves into a specialist. Specializations enable better cohabitation and utilization of the partner while inflicting less damage. Even parasites aim to reduce damage done to their host in a proverbial effort to not bite the hand that feeds them. Some parasites, however, have specifically adapted to kill their host as part of their occupation. These parasitoids, such as several wasp and fly species whose individual eggs are deposited into ant or spider hosts, must therefore maintain rates of reproduction that avoid the depletion of their host species. The threat of over-exploitation of hosts is likely a factor in the relative abundance of parasitic species compared to parasitoids, which would be at least secondary consumers.

Likewise, a host usually outnumbers its parasite. Even so, plant hosts can defend themselves. Methods of chemical or physical defense, such as bitter alkaloid compounds or tough spines, repel parasites before either proactively or retroactively. Unfortunately for most host plant species, the generation times of their insect parasites are usually considerably shorter

than their own, allowing for uneven rates of evolutionary advancement. Therefore, the parasites have an advantage, and some have developed fascinating methods of exploitation.

Cynipid wasps (Hymenoptera: Cynipidae: Cynipini) are parasites of several plant and insect taxa. One of the most common and easily recognized host groups is the oaks (genus *Quercus*). Once adult females have deposited their broods, the eggs or larvae secrete growth-regulating hormones to induce the plant to produce specialized growths, called "galls." Galls act as long-term nurseries for the developing young, with some species taking up to three years to emerge (Hodges *et al*., 2006). Galls may be single- or multi-celled, and may contain only one or several larvae per cell. Cynipid wasps lay their eggs on host plant stems, leaves, veins, buds, catkins, or roots, depending on Cynipid species (Abrahamson & Weis, 1987). Gall structures are diverse and highly specific. Various internal and external structures have developed as protection against predators, parasitoid wasp species, or inquilines looking for a gall to commandeer (Bailey *et al.*, 2009).

In additional to their specialized niche, Cynipids have evolved cyclically parthenogenetic life cycles, adding to the structural diversity of the galls produced (Stone *et al*., 2002). While the all-female asexual generation may produce one type of gall on a certain area of the host species, the sexual generation can diverge from the latter by gall type, location, or even host species (Hodges *et al*., 2006). Furthermore, asexual and sexual females of the same species do not resemble each other. This reproductive strategy, heterogony, has caused considerable confusion in the taxonomic classification of entire genera. While only one generation of some species are known, it is likely that several described species are simply the sister generations of others.

Making classification more cumbersome is the wasps' short time outside the gall. While full life cycles can last as long as three years, the larvae's developmental stage consumes the vast

majority of a Cynipid's lifespan. Adults are not known to feed. New adults seem to only emerge from their galls to reproduce, and subsequently die. Therefore, the wasps live most of their lives as parasitic larvae, feeding on the sugars and nutrients provided by their host oak. Many Cynipid species emerge in the spring, with their galls maturing and becoming more distinct in fall and winter. Cases of severe infestation are rare, but weakened trees have been known to succumb to high densities of galls.

Over 1,360 species of gall wasps have been described (Buss, 2008). However, they garner little public or scientific attention. This disinterest is likely a derivative of what the wasps lack in stature—adult wasps only measure from 1 to 6 mm in length, and larvae only 1 to 4 mm (Buss, 2003). Add on the rarity of encountering an adult outside the gall, and even a highly-trained naturalist would swat a Cynipid away as a common gnat. The galls themselves are often camouflaged or inconspicuous, and therefore do not draw the eye of a casual observer. Even further, their absence in many urban areas due to lack of host plants or pesticide use has kept their galls from the public eye.

While Cynipid specificity in hosts helps keep gall wasps under the radar, it is not an unintentional adaptation. The deeply-ingrained predilection of some genera for oaks takes advantage of their high tannin levels. Tannic acids, used in particularly high concentrations by oaks, have anti-bacterial and anti-fungal properties that protect the gall just as much as the tree (Taper & Case, 1987). Dense fungal endophyte populations within the host plant are associated with high mortality rates in Cynipid larvae (Wilson & Carroll, 1997). Even further, tannins act as astringent herbivory deterrents (Forkner *et al*., 2004), and several galls are known to induce the production of excess acid, likely to stave off predation. Certain biochemical or physical defense adaptations can therefore benefit both the host and the parasite.

Conversely, oak hosts do not seem to have any significant defenses against their Cynipid parasites. The collection of specializations developed by Cynipids and the relative lack of defenses in oaks suggest uneven rates of coevolution. There is little to stop Cynipids from penetrating the bark, leaves, and roots of their hosts, whether by long ovipositors or burrowing larvae.

The specifics of the interactions vary by species, and are often affected by regional conditions. Florida houses a spectrum of plant communities that range from seasonally hydric to xeric, and consequently houses a large diversity of oaks. Upland communities, including sandhills, clayhills, flatwoods, and scrub communities all house several *Quercus* species. These oaks often cohabitate, and therefore have partitioned niches within their optimal ranges (Cavender-Bares *et al*, 2004). The typically dry, sterile, sandy soils within these communities have produced many other specialized plant taxa as well, from shrubs and grasses to large pines (Ewel & Meyers, 1990).

Notable upland oak species include the live oak (*Q. virginiana*), sand live oak (*Q. geminata*), laurel oak (*Q. laurifolia*), Chapman's oak (*Q. chapmanii*), myrtle oak (*Q. myrtifolia*), scrub oak *(Q. inopina)*, bluejack oak (*Q. incana*), and turkey oak (*Q. laevis*). The smaller, hardier *Q. geminata*, *Q. chapmanii*, *Q. inopina*, and *Q. myrtifolia* are typically found in drier areas with higher burn frequencies and must regrow from their roots if destroyed in a fire. *Q. virginiana*, *Q. laurifolia*, *Q. incana*, and *Q. laevis* often grow much larger and are found in areas of lower burn frequencies, though their growth also depends heavily on the surrounding soil moisture. The latter group generally prefers more fertile soils, and therefore likely hosts more species of Cynipids (Blanche & Westoby, 1995). At least 79 species of Cynipids form galls on the eight oak species listed, though more are expected to exist (Cornell, 1986). The galls are most

commonly formed on the leaves and stems of these oaks, though bud, catkin, and root galls are also included. Some Cynipid species parasitize several of types of oaks; other Cynipid species are more host specific. The Cynipids can distinguish between the oak species by recognizing unique chemical compositions of their hosts, and can even distinguish between hybrids (Abrahamson *et al*., 2003).

Cynipid species, therefore, are closely tied to the variables which limit the geographic range of their hosts. This has offered opportunities for the development of regionally endemic species, and has contributed to the emergence of biodiversity and richness hotspots in much of Florida upland, flatwood, and scrub communities (Price *et al*., 2004). However, relatively little is known about the taxonomy and natural history of Cynipid gall wasps, including the extent of their role in their communities. Further study and understanding of these species and their interactions with their niches is vital for both proactive and restorative conservation initiatives for their habitats. By comparing the distributions of Cynipid galls along trails and roads with those a short distance away, the edge effect of those pathways can be measured. The verification of any edge effect will allow for either easier or more accurate sampling in future surveys. Should there be no significant edge effect, sampling of Cynipid galls could be completed directly from trails or roads. However, should there be a significant edge effect, future sampling should be completed off-trail, in areas away from the influences of any pathways.

This study aims to both add to the current understanding of the interactions between Cynipids and their communities, as well as to better inform future sampling methods when conducting research in this field.

Introduction

The "edge effect" describes the changes in a community's structure at its boundary with another community. In many cases, these boundaries are areas of high biodiversity (Harris, 1988). Residents of both communities are able to cohabitate in this transitional zone, where the gradients of the two communities blend. Such transitional zones, or ecotones, have become a topic of study over the last century. Early focus of transitional zone study was on game management; current focus is on plant and ecosystem conservation.

However, the edge effect is not restricted to the shared boundary of adjacent communities; it can also be seen where communities are disrupted by anthropogenic roads, walkways, developments, and other structures. In these cases, the boundaries of the natural communities are much more distinct, and may even lack any notable transition zones. Whereas natural community boundaries can stretch out over large distances, these abrupt anthropogenic boundaries often mark complete and sudden alteration of community structure. The extent of the effect of abrupt anthropogenic boundaries on the natural areas immediately adjacent to these boundaries is not fully understood, especially potential changes in the insect members of a disrupted community.

Variables such as sunlight penetration, temperature, and wind speed in the immediately adjacent area can be altered by distinct boundaries bordering areas of bare ground as small as recreational trails. While any of these possible alterations might be slight, they can still influence both stationary and small members of a community. If light and temperature conditions along the edge of a path vary from those conditions a short distance away, some plant species might not survive as well nearer the path. Thus, small differences in the distribution of plants between the

two areas might lead to large differences in the distribution of animal species that rely on the plants. In short, if the distribution of oak species assemblages differs on and off the path, Cynipid wasp gall distribution should also differ.

Currently, surveys and data collection practices require surveyors to move off anthropogenic pathways to gain as precise an understanding of the community as possible. If we find that variation—or lack thereof—in conditions on and off pathways have no significant effect on the distribution of oaks or Cynipid, then future botanical and entomological surveys could use pathways as sampling transects. The advantage of using anthropogenic pathways would make data collection easier for both researchers and citizen scientists, and could spare sensitive areas from damaging foot traffic. While the findings of this study cannot be generalized to other genera of plants or animals, they can be utilized during future studies on this unique family of wasps.

This study will attempt to add to our collective knowledge of the Cynipid species of southwest Florida, as well as provide evidence to either support or refute the generally held notion that sampling should be done away from any trails or roads. Subsequent studies within similar areas will therefore be better informed as the most accurate sampling methods.

Methods

Study areas: Theoretically, the methods used in this study could be applied to any ecological community, as long as it has some distinct edge. For Cynipids and oaks, however, oak species richness and height provide limitations. If only one or two species of oaks are found within the sampling area, then the only Cynipid species found will be those adapted for that host species.

Any data and conclusions would therefore be limited to a small set of genera, which is instrumental in understanding individual species' natural histories, but not for understanding community interactions through a wider lens. At the same time, should the oaks being sampled be tall enough to reduce the ability to see and handle the galls, accurate identification and population counts would be impossible from the ground.

Therefore, the need for several species of short oaks in a single area drives the choice of sampling locations for studies on Cynipid galls. Florida, by way of its sandy soils and frequent natural burns, has fortunately developed several such areas. While most pine communities contain plenty of oak species, scrub and scrubby flatwoods largely maintain them at the perfect heights, either by aridity or more frequent burning. Species such as live oak (*Q. virginiana*), sand live oak (*Q. geminata*), laurel oak (*Q. laurifolia*), Chapman's oak (*Q. chapmanii*), myrtle oak (*Q. myrtifolia*), scrub oak *(Q. inopina)*, bluejack oak (*Q. incana*), and turkey oak (*Q. laevis*) frequently inhabit these two communities (Ewel & Myers, 1990). Some of these species can grow relatively large in scrub and scrubby flatwood communities, but large individuals are usually not the majority. Therefore, these two natural communities are ideal for Cynipid gall distribution studies in Florida.

Little Manatee River State Park is a 2,418-acre Florida State Park located in southern Hillsborough County. The park boundaries extend to the northeast and southwest of the 4.5 mile stretch of the Little Manatee River that flows west across it. The first parcel of what now constitutes Little Manatee River State Park was purchased by the state in 1974. Portions of the park were used for cattle grazing and pine production prior to acquisition by the state. These uses suggest natural burns were suppressed for a significant portion of the area's recent history. Fire suppression can have over-reaching effects on plant community composition for several years

afterwards, and can allow for entire community shifts (Menges & Hawkes, 1998). However, land management practices that encourage restoration of historic communities are now widely practiced within the state's natural parks.

According to the Little Manatee River State Park 2004 management plan, there are thirteen ecological communities within the park: mesic flatwoods, sandhill, scrub, scrubby flatwoods, upland mixed forest, xeric hammock, baygall, depression marsh, dome, floodplain forest, hydric hammock, blackwater stream, and ruderal/developed areas (see Figure 1). Within these communities are also several gradients of soil types, most of which are composed primarily of shell or quartz sand. Sand-based soils are extremely barren in respect to nutrient and water retention. Hydronium ion inundation from surrounding pines adds to the sterility of these sands. As a result, scrub and flatwood plants have become specialized survivalists, employing strategies ranging from thick, waxy leaves to dependence on fire for seed germination. Many of these species have become endemic to these communities, and as a result have increased species richness and diversity. In flatwood communities, 100 species of groundcover plants can occur in a single acre, though are most evident following a burn (Ewel & Myers, 1990). These burns maintain a fairly open understory, allowing many shrub and grass species to thrive. Scrub communities rely less on fire and more on xeric conditions to control understory growth, but do not support grasses as flatwoods do. Light conditions can vary in scrubs, but, in general, only short pines and oaks threaten to reduce sunlight penetration. At the same time, if left unburned the oaks can overgrow the underbrush and reduce light available for shrubs and grasses. They do not always perish in fires, and many regrow from their roots to form small clonal stands. Both their partial resistance to infrequent burns and their communities' susceptibility to reduced sunlight requires that oaks be given high priority in management plans.

Alongside their wide collection of resident plants, scrub and flatwood communities generally have just a few dominant species. These include sand pine (*Pinus clausa*), sand live oak (*Quercus geminata*), Florida rosemary (*Ceratolia ericoides*), and saw palmetto (*Serenoa repens*) for scrub and slash pine (*Pinus elliotii*), longleaf pine (*Pinus palustris*), various *Lyonia spp.*, and wiregrass (*Aristida stricta*) for flatwoods. It should be noted, however, that flatwoods do have greater variation in dominant species compositions than scrub.

The collection of oak species previously mentioned as being abundant in scrub and scrubby flatwoods communities are rarely among the dominant species, with the exception of *Q. geminata* in scrubs. However, these oaks are still represented in significant numbers and should be noted for their diversity within these communities. They are adapted to handle the acidic, dry (or occasionally saturated), sterile conditions, and have established themselves as integral providers of food and shelter for many groups of fauna. This of course includes Cynipid wasps, which have evolved into exquisite examples of complex parasitism. Without further research into these relationships, future research and conservation opportunities may be lost.

Sampling methods: To compare gall distributions on and off trail, I took a pair of parallel sister transects in each of five locations within Little Manatee River State Park. Each transect was 20 meters long and covered an area 1 meter wide and 2 meters high. While one transect of each pair ran along the edge of the trail, the other ran 10 meters off the trail. Any galls within the transect sampling areas were recorded and/or photographed, and identified either on- or off-site. Unknown species were given a numeric identifier and recorded. I took the five transect pairs from scrubby flatwood communities within the park (Fig. 1b). Sampling took place from December 11 to December 24 of 2016.

The data points collected for each gall included the Cynipid species, host tree species, and height at which it was found. Height was measured in 0.25 meter intervals in an effort to conserve time. Each type of gall found was photographed and measured using a gridded index card placed directly behind the gall. These photographs both visually represent the species observed and aid in species identification when not possible on-site. Identification was done through the consultation of guide books—see Hodges *et al.*, 2006—online guides from university, government, or institutional collections, and contact with Archbold Biological Station's Entomology Department. There is a total of seven unknown species of galls recorded in this study (Table 2), which likely are results of a lack of accessible identification tools. However, it should also be noted that Cynipid species assemblages are not complete (Cornell, 1985).

Before collection of any data from the transects at each site, I also recorded site characteristics (Table 1). This includes thickness of any loamy build-up and prominent plant species. Particular emphasis was put on the presence of pines and multiple oak species, including whether any oaks were large enough to withstand an ecological burn. These characteristics were recorded due to the considerable effect that pines have on soil acidity and the probable diversity in Cynipid species in the presence of multiple oak species. Older oaks can also explain variation in distribution due to old galls having remained on their hosts. It is important to note that regular burning does not harm the Cynipid community (Siemann *et al.*, 1997), but can destroy galls on low-lying trees and disrupt sampling.

In addition to these biotic characteristics, the sites' abiotic characteristics were noted as well. These included any topographical sloping or interruptions along transects and the brush density of the sample site. Any sloping of the sampling area could indicate a moisture gradient along the transect or between the sister transects, which could affect the vegetation and gall

distribution (Blanche & Westoby, 1995). The proximities of the sampling sites to the Little Manatee River were also noted. These site descriptions can, at least in part, explain differences seen in species assemblages between sister transects and sample sites. However, as these factors were not measured with precise methods or instruments, they can only stand as observations amongst the data. Further analysis with these factors would require more accurate measurement.

I used JMP 13 Statistical Software to analyze and compare the data sets collected. Through JMP 13, I ran ANOVA, Pearson, and fit model analyses to quantify the level of connectivity between the sister transects and the different sampling sites. The seven unknown species were excluded from all analyses to prevent any possible infiltration by other galling insect species. My working hypothesis for this study was that the presence of the recreational trails will have a significant effect on the distribution of Cynipid galls. I tested my hypothesis using a fit model to compare the influence of the trail to the other major influences on gall distribution. Because host specificity (Fig. 4), variation in species frequencies across sample sites (Fig. 5), and preference for different altitudinal ranges (Fig. 6) were all shown by the Cynipids, these three variables were included in the fit model analysis.

Quantifying any edge effect present in this community will be informative for future research in gall ecology that requires an understanding of gall distribution in order to select unbiased sampling areas. Further, it is likely that some species of both *Quercus* and Cynipidae exhibit varying levels of tolerance the conditions along the edge, and will therefore fare differently along the pathways. While that still entails an altered community structure, it could mean potentially higher gall frequencies along footpaths, increasing visibility and accessibility for researchers. Therefore, understanding the "edge effect" introduced by recreational trails can inform both distribution studies and species surveys.
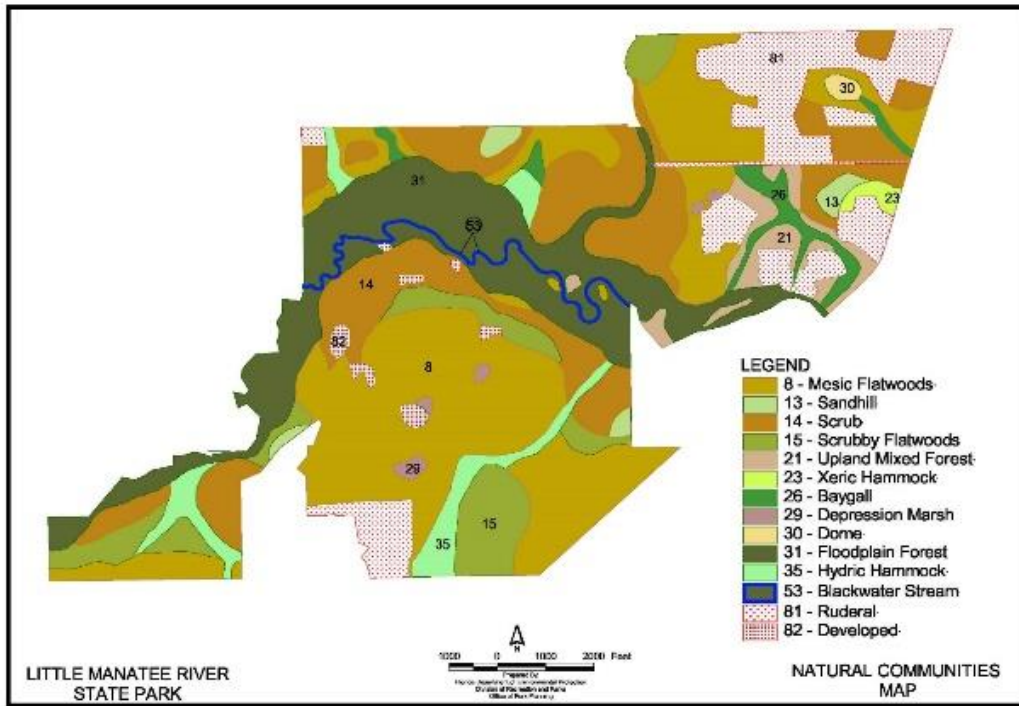
Figures

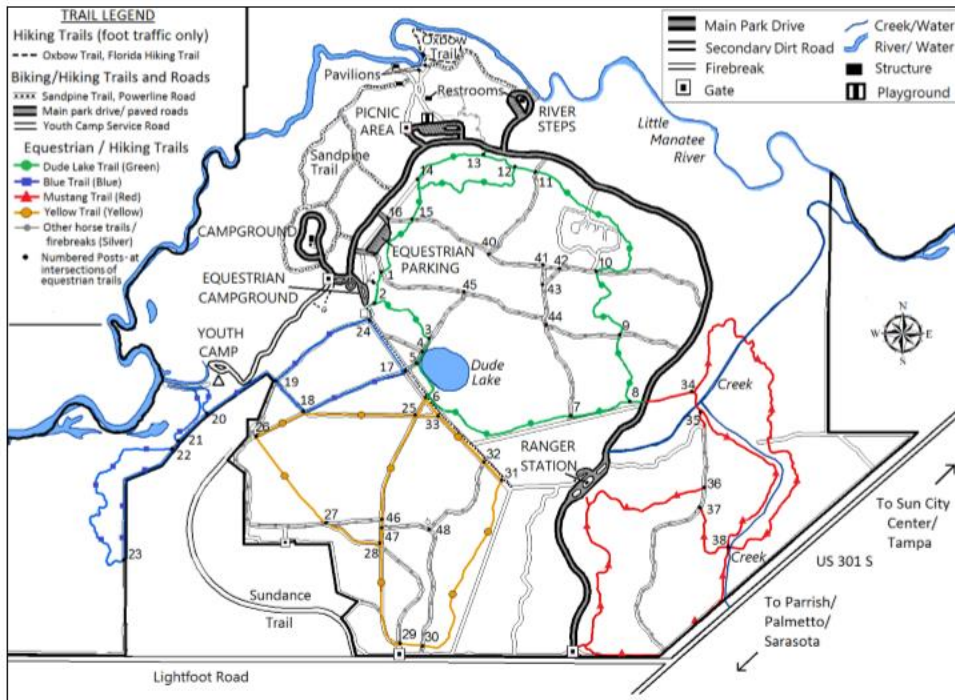**Figure 1a**   LMRSP Natural Communities Map.

Figure 1b   Little Manatee River State Park southern trail map.
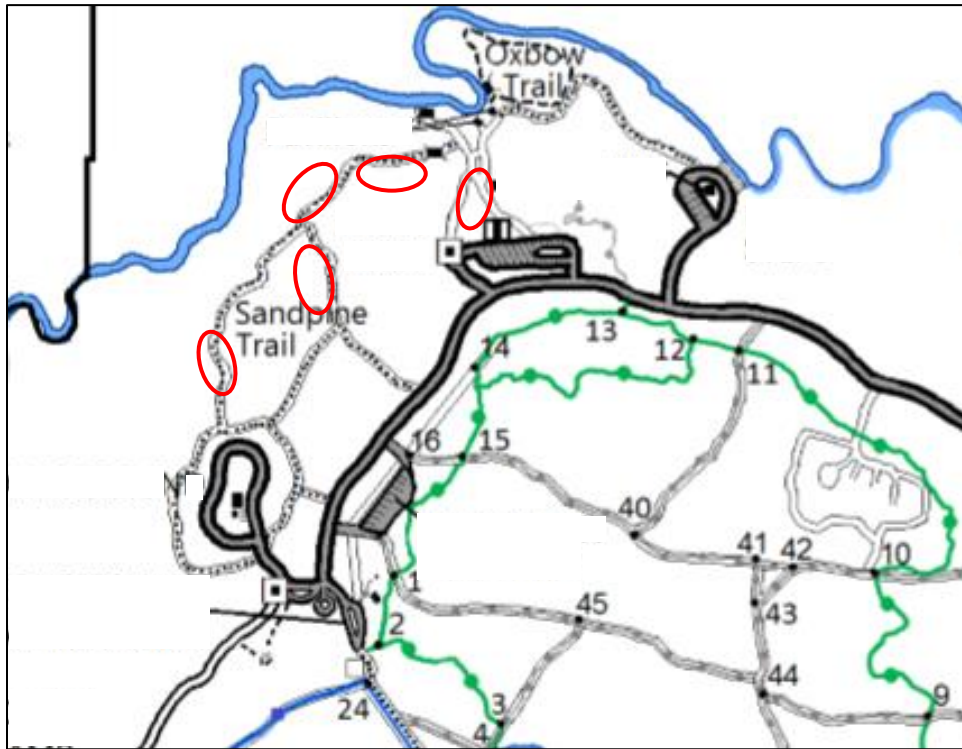
Figure 1c   Sample site locations within in the scrub and scrubby flatwoods of LMRSP.

Note: Sample areas highlighted in red.

Results

A. quercusfoliatus

A. quercuslanigera

B. quercusvirens

C. quercusbatatoides

C. quercussimilis

A. murata

D. quercusvirens

E. floridana

D. nova

A. gainesi

D. quercusverrucarum

Figure 2a   Images of identified Cynipid galls from sample sites.

Note: Photographs of Andricus cinnamoneus *and* Neuroterus quercusminutissimus *are not included above due to loss of on-site images.*

| Unknown sp. 1 | Unknown sp. 2 | Unknown sp. 4 | Unknown sp. 6 |

| Unknown sp. 7 | Unknown sp. 8 | Unknown sp. 9 |

Figure 2b Images of unidentified galls from the sample sites.

Table 1 Physical site characteristics & dominant plant species present.

Site	Plant Density	Sloping	Large Quercus (>2 m)	*P. clausa*	*A. stricta*	*S. repens*	*O. americanus*	*Lyonia* spp.	*X. americana*
1	High	N	X	X		X	X		X
2	Moderate	Y		X	X	X	X	X	X
3	Moderate	N	X	X		X	X	X	X
4	Low	Y	X	X		X		X	X
5	High	Y		X	X	X	X	X	

17

Of the 3,339 galls recorded, 76% were known. Four species comprised 81% of the known

species (Table 2) and 57% of all known galls were found off the trail (Fig. 3: n = 2546).

Table 2 Total Observed Cynipid Galls

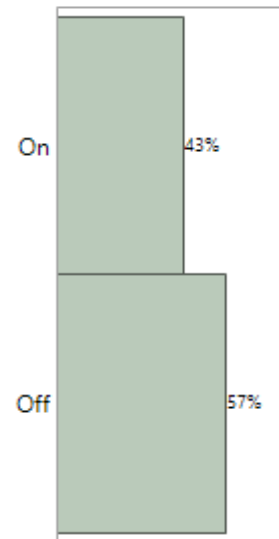| Cynipid Species	Count	Frequency
1	186	0.0557
2	41	0.0123
4	106	0.0317
6	421	0.1261
7	15	0.0045
8	3	0.0009
9	21	0.0063
Amphibolips gainesi	1	0.0003
Amphibolips murata	70	0.0210
Andricus cinnamoneus	66	0.0198
Andricus quercusfoliatus	98	0.0294
Andricus quercuslanigera	8	0.0024
Belonocnema quercusvirens	291	0.0872
Callirhytis quercusbatatoides	98	0.0294
Callirhytis quercussimilis	327	0.0979
Disholcaspis quercusvirens	548	0.1641
Eumayria floridana	21	0.0063
Neuroterus quercusminutissimus	117	0.0350
Neuroterus quercusverrucarum	901	0.2698
Total	3339	1

Figure 3 On/Off Trail Frequencies

Each Cynipid species showed significant preferences for one or more *Quercus* host species (Fig. 4; Pearson: $\chi^2 = 0.938$, p < 0.0001). Variability in preference of host species was low.
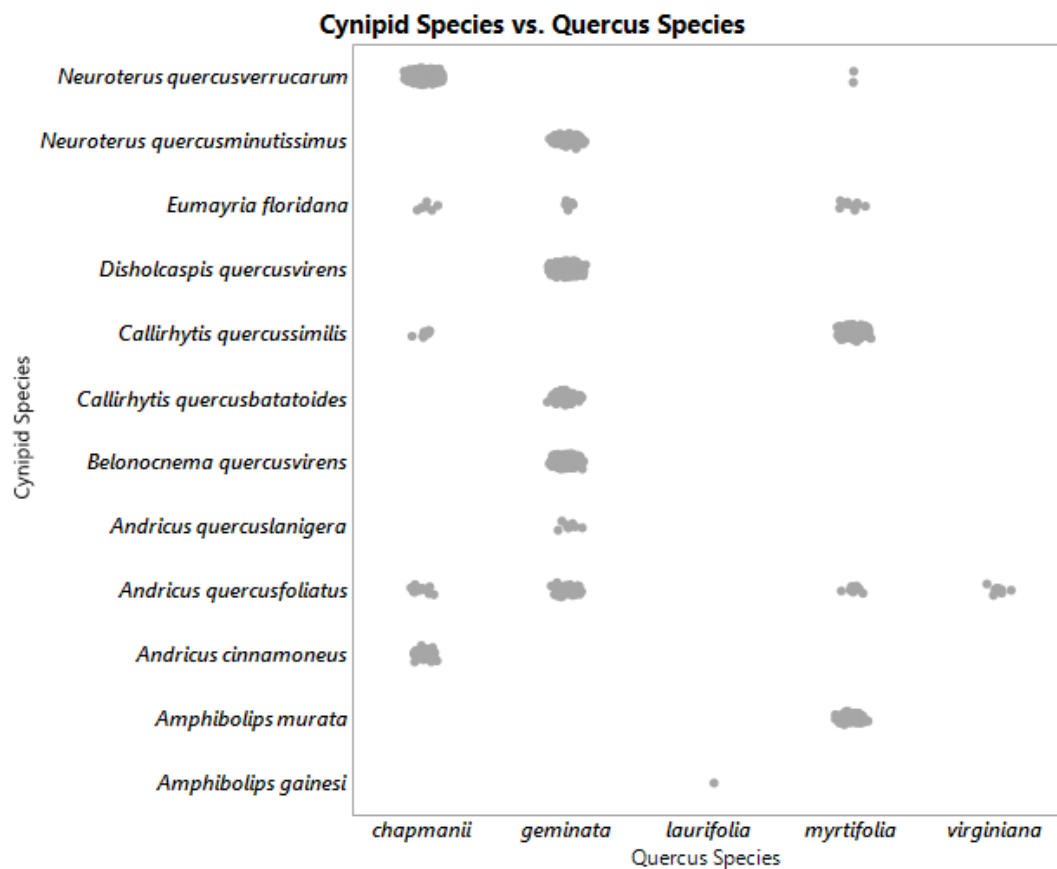

Figure 4 Distributions of Cynipid galls across *Quercus spp*.

Galls were found at significantly different frequencies between the five sample sites (Fig 5; Pearson: $\chi^2 = 0.204$, $p < 0.0001$). The variability in ovipositioning preference between sample sites was high.
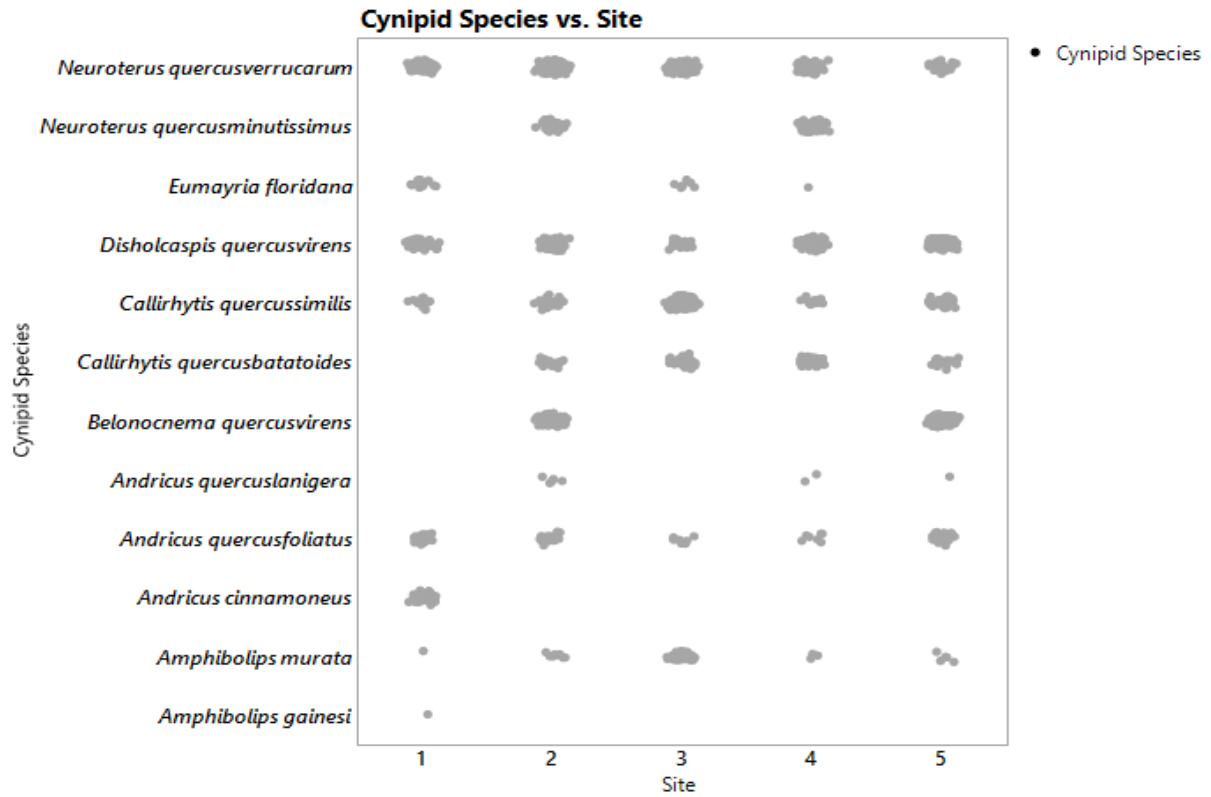

Figure 5 Distributions of Cynipid galls by sample site.

The different Cynipid species were found at significantly different heights on their host trees (Fig. 5; ANOVA: $\chi^2 = 0.157$, $p < 0.0001$). Mean gall height equaled 1.18 meters.
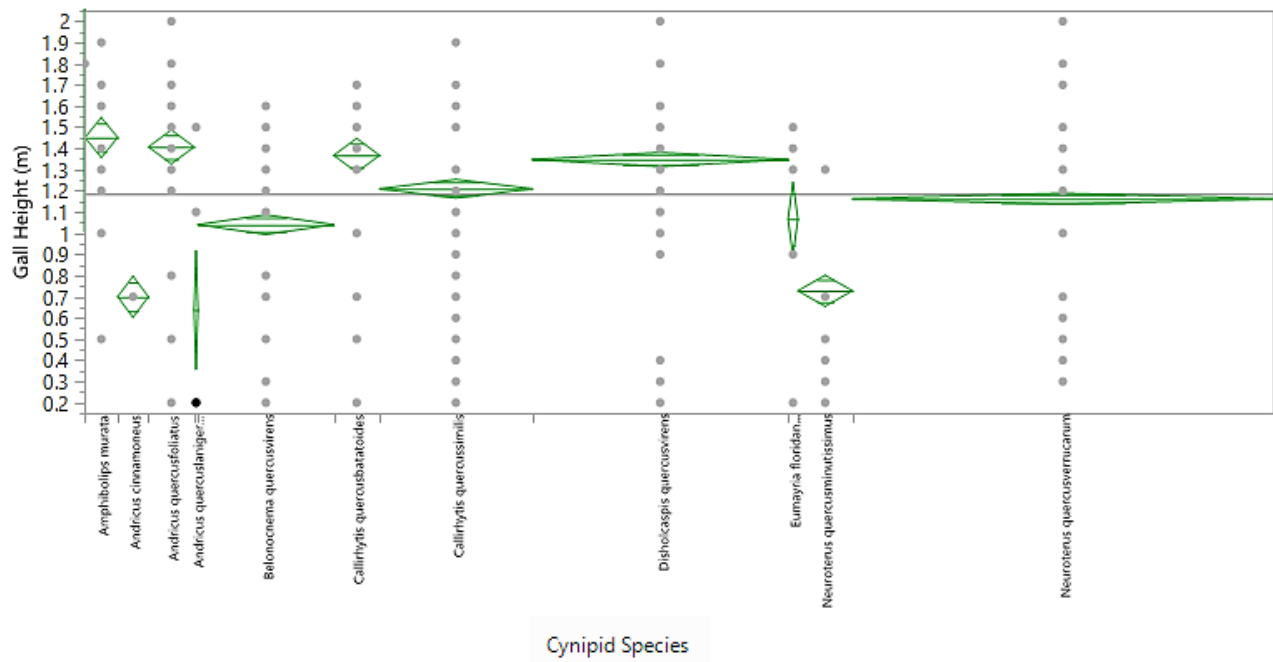

Figure 6   Distributions of Cynipid galls by their height.

There was a significant difference in species preference in ovipositioning sites based on survey transects on the trail and 10 meters off the trail (Fig. 4; Pearson: $\chi^2 = 0.041$, $p < 0.0001$). However, variability in female preference of ovipositioning sites was high.
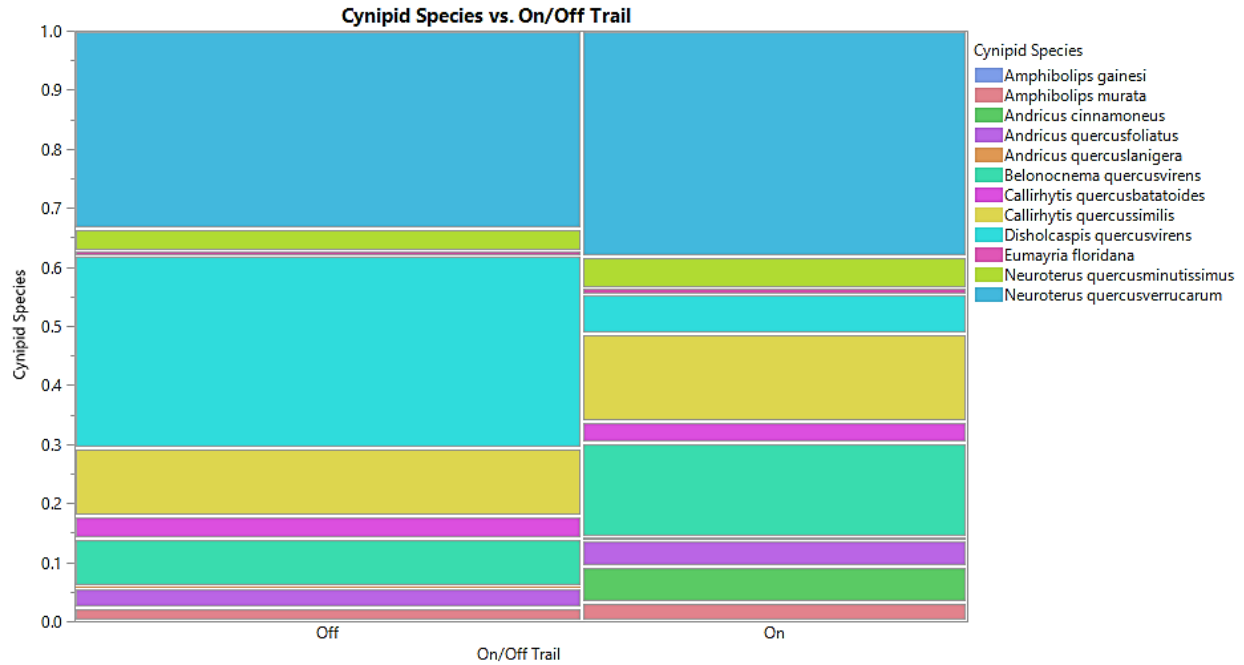

Figure 7   Distributions of Cynipid *spp.* relative to five sample trails.

The species of oak trees accounted for 81% of variation in oviposition sites by Cynipid females were the species of oak trees (Table 3). The area sampled, gall height and the edge effect accounted for the remaining 19% of variation in the decision of females to deposit eggs.

Source	LogWorth	% Effect
Quercus Species	1540.635	81.25
Site	188.364	9.93
Gall Height	89.123	4.70
On/Off Trail	78.075	4.12
Total	1896.197	100

Table 3   Sources of variation in oviposition sites selected by Cynipid females.

Discussion

The most accurate predictor for Cynipid distribution was which Quercus species were available in the sample areas. The ties between Cynipids and their hosts have been well documented and is a standard principle in the study of gall wasp ecology (Abrahamson *et al*, 2003) (Blanche & Westoby, 1995) (Price *et al*, 2004). Host species preference was also the most effective at explaining any variability amongst the data, producing the highest R^2 value of all tests (Fig. 4).

Distributions also varied by sample site and the height at which each gall was found, but both had high variability (Figs. 5 & 6). Factors such as brush density, host density, soil moisture, and light penetration could vary by sample site and can influence ovipositioning rates. As for why the different Cynipids preferred different altitudinal ranges of their hosts for oviposition, there are several possibilities. New hatchlings may not stray far from their home galls in search of oviposition sites, eliminating interspecies competition and creating multigenerational zones of infestation on each host. This explanation, however, would be less advantageous for the sexual generations of Cynipids, as it would encourage inbreeding. Alternatively, some species may outcompete others for the areas of young growth needed for anchoring eggs, effectively creating micro-niches on each host. However, each species is usually confined to one type of host tissue it can parasitize, reducing the frequency of interspecies competition. Subsequently, species that are confined to new shoot growth will be limited to altitudinal ranges which produce the most new shoots in springtime. The same process would limit catkin, bud, and leaf gallers alike. Because fire regimes keep height variability low in the sample sites, these areas of specific growth would fall within similar altitudinal ranges throughout each site. Each Cynipid species, therefore, would appear to have an altitudinal preference for oviposition.

When the data from the five sample sites were combined, a slight preference for oviposition sites off the trail was revealed (Fig. 7). Fifty-seven percent of all galls were found off trail (Fig. 3). However, variability within the data was so high that other factors had substantial effects on distribution. Host densities, brush density, accessibility for predators, and other variables could vary on- and off-trail. Oaks off the trail could also have slightly older age ranges due to maintenance along the trail, possibly providing more habitable space for the Cynipids.

The final analysis of my data was with a fit model to quantify the effect each main variable had on the distributions of the Cynipid species (Table 3). By finding the percent frequency of each variable's LogWorth values within the model, I calculated the percent total effect on the distributions. My null hypothesis, to test for the presence of any edge effect, was that there would be no significant difference in preference of oviposition sites between the on-trail transects and those 10 meters off the trail. As shown in Figure 8, approximately 4% of Cynipid species distribution could be explained by their position relative to the trail. Therefore, there is not a significant edge effect along the trails and I cannot reject my null hypothesis.

When gall distributions are analyzed through their positions relative to the trail alone, there is a significant difference. However, when all other recorded factors are included in the fit model analysis, the total effect of the trail is reduced to an insignificant percentage (Fig. 8). This conflict likely lies within the high variability of the on/off trail data. My conclusion is that because there is not a significant edge effect, anthropogenic trails can be used as unbiased sampling transects for future Cynipid gall surveys and research. However, this relies upon the trail edge having the same species, age ranges, and densities of hosts as the interior community. The edge, in itself, will not affect distribution, but the variations that can come with it will.

It should be noted that other statistical analysis methods may better account for the high variability within my data. The two contradictory results in Figures 7 and 8 could be reconciled through these other means. Therefore, further analysis is required before my conclusions can be applied in future research.

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