

**BEYOND FOLIVORY: THREE TYPES OF INTERACTIONS BETWEEN PLANTS AND  
INSECTS IN THE PINE BARRENS COMMUNITY OF LONG ISLAND, NEW YORK**

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## ABSTRACT

In our research, we have focused on three types of plant-insect interactions that have received less attention than Folivory: the interaction of Cynipid wasp and oak galls, the secondary community found in abandoned galls, and the effect of Allegheny mound ants on surrounding vegetation. First, the patterns of growth, photosynthetic capability, and mortality patterns of the galls were examined. The fresh galls were taken to X27A beam line at the National Synchrotron Light Source (NSLS) to analyze the elemental concentrations of the gall and leaf. The results showed high concentration of Mn (from the soil) in both leaf and gall. Second, we studied the secondary community of abandoned galls which, after the wasps emerge, then become a refuge for ants and spiders. Abandoned and fresh galls were collected twice a week from the field. We dissected them under the microscope at 40X and have identified the ants and spiders in the galls to the family level. In addition to these two gall interactions, we examined the relationship of Allegheny mound ants with plants. Ants aggressively keep their mounds free of plants to maintain high temperature for the development of their eggs. Using store bought annuals, we demonstrated 100% mortality of *Petunia*; when plants were planted on the ant mound and death zone. However, we observed healthy plants (bear berry and Pennsylvania sedge) living on the mound. We are interested in this marked pattern of ant-induced plant death and why bear berry and Pennsylvania sedge seem to escape the attack. The goal of this research is to study how plants and insects interact in the pine Barrens beyond Folivory (eating of plants) and be familiar with the natural history of the gall-makers and from this generate a testable hypothesis for future research.

## INTRODUCTION

Plant and insects are omnipresent in terrestrial ecosystems. Because of the vital role that insects play as herbivores, most plant and insect ecology studies focus on that interaction, particularly 'Folivory'. Plants and insects interact continually in complex ways [1]; and these interactions can range from symbiotic to parasitic. The interaction between plants and insects is dynamic and it is subjected to continual variation and change. Plants developed different mechanisms to reduce insect attack including specific responses which alter their chemical and physical aspects. On the other hand, insects developed several strategies to overcome plants defense barriers, allowing them to feed, grow and reproduce on the host plant [2].

The gall, the secondary community of abandoned galls, and the Allegheny mound building ants were the three types of plant and insect interactions studied in this research. Galls are plant tissues, induced by another organism, which provides that organism with food and a measure of physical protection [3]. Galls are formed in response to eggs laid on scrub oak tree (*Quercus ilicifolia*) by Cynipid wasp (*Amphibilops ilicifoleae*). The process of gall induction on the scrub oak is a parasitic relationship. In other words, all cynipid wasps are parasites of the scrub oak. Evidence, nevertheless suggests that gallers are parasites that control most aspects of the gall development. Galls do provide some protection against attack by non specialist predators but they are far from being enemy free space, most are attacked by fungi, parasitoids, and natural enemies that often inflict high mortality [4]. The female wasp oviposits on scrub oak during May and June the gall begins to form; inside the gall lives the larva of the wasp. It feeds on the gall tissues and continues to develop until it reaches adult stage, then the wasp chews its way out and emerge during July and August. Aside from the gall maker and host plant, there are many

other organisms involved in this complex interaction. Parasites and inquilines can infect a developing gall, killing the wasp larva, therefore increasing mortality rate. The second type of plant and insect interaction we studied in this research is the secondary community. As soon as the wasp emerge through the exit hole, ants, spiders, caterpillars etc. use the abandoned gall as a refuge.

We also studied the interaction of Allegheny ants with plants around their mound. Allegheny mound ants (*Formica exsectoides*); is found in the Pine Barrens forest, because the soil is well drained and there are a lot of open spaces [5]. Members of this family are mostly black, brown or reddish and they have a complex social structure usually consisting of sterile females and winged fertile males. After mating, the male dies and female lose their wings and return to the ground to start a new colony [6]. They feed on other insects including wasp for protein and Aphid honey dew for sugar. The mounds are constructed of soil brought up from excavated galleries below the nest. This ant attempts to kill any vegetation that may grow on the mound or shade the area except Bear Berry and Pennsylvania Sedge. They bite, deposit formic acid into the wounds and kill most plants on or around the mound including scrub oak plants. They build the mound in undisturbed open areas; a 19 inch mound may contain 250,000 workers and over 1,000 egg laying queens [7].

We have observed that these three types of plant and insect interactions in the Pine Barrens forest are interrelated. These observations lead to many questions. What type of chemical does wasp secrete on scrub oak tree to form a gall? How are galls formed? Does gall have any negative effects on scrub oak tree and does it have any economic significance? What is the rate and agents of mortality of Cynipid wasp? What makes Bear Berry and Pennsylvania sedge resist attack by Allegheny ants? Little or no work has been done on the Cynipid wasp gall in the Pine Barrens

forest of Long Island. The only work done on cynipid gall was in 1876 by Lewis H Weld; he observed that the gall has high mortality rate 95%. Therefore, The goal of this research is to study how plants and insects interact in the Pine Barrens beyond Folivory (eating of plants)

## **MATERIALS AND METHODS**

The gall and abandoned gall studies were conducted in summer of 2010 in the Dwarf Pine Barrens (DPB) of West Hampton Beach, Long Island, New York. The DPB are defined by the dwarf form of pitch pine (*Pinus rigida miller*). It is a globally unique habitat made up of dwarf pines, and Ericaceous shrubs. A scrub oak rarely exceeds 3m in height and form a shrub layer. The scrub oaks in this area had little or no canopy.

In order to study the secondary community of abandoned galls, we went to the forest twice a week; we collected both fresh, abandoned galls and oak leaves. They were brought to the laboratory and kept in a refrigerator for few days. The abandoned galls were dissected under the microscope at 40X magnification. The critters were placed in vials with 70% ethanol (C<sub>2</sub>H<sub>5</sub>OH). We attempted to identify the critters; measured the abandoned gall's length, width including the exit hole size and recorded the data for further analysis.

The fresh galls and leaves were taken to National Synchrotron Light Source (NSLS) at X27A beam line to estimate the elemental concentration and their distribution in the various types of tissues in the galls and leaves especially Manganese (Mn) and Rubidium (Rb). The fresh galls and leaves were embedded in optimal cutting temperature embedding medium and cryotomed to 30µm thick at -12°C. The cross sections were mounted on ultralene (an x-ray transparent material). Data processing was performed using the beam line software. Synchrotron-based X-ray micro fluorescence (µSXRF) analysis of Scrub Oak leaf and gall tissue thin sections

(20  $\mu\text{m}$ ) was performed at Beam line X27A of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (Upton, NY) [8]. Briefly, this beam line uses Kirkpatrick-Baez (K-B) mirrors to produce a focused spot (7 by 14  $\mu\text{m}$ ) of hard X-rays with tunable energy achieved via Si (111) or Si (311) channel-cut monochromator crystals. For  $\mu\text{SXRF}$  data acquisition, the incident energy was fixed at 17 keV to excite all target elements simultaneously. Samples were positioned in the path of the beam by an XYZ stage oriented 45° to the beam, and X-ray fluorescence was detected by a 13-element Canberra Ge array detector positioned 90° to the incident beam. Energy dispersive spectra (EDS) were collected for 300 seconds at various points of interest which were chosen by identifiable anatomical features observed with the beamline optical microscope camera. Data acquisition and processing were performed using IDL-based beamline software designed by CARS (U. Chicago, Consortium for Advanced Radiation Sources)

Store-bought annuals (*Petunias*) were planted on the Allegheny mound ant for mortality and behavioral studies. Three mounds were selected randomly and a total of 54 *Petunias* were planted. Six *Petunia* plants were planted on the ant mound (AM), zone of death (ZD) and outside the mound, respectively. The ants response to the *Petunias* planted on their mound was observed twice a week. We also planted Pennsylvania sedge and on 5 ant mounds, they were watered and observed twice a week as well.

## RESULTS

### Galls:

It has been observed that galls has high mortality rate, and the agents of mortality were identified to the family level; they are Eulophidae, Torimidae and Perilamphidae [ figure 1].



At the National Synchrotron Light Source (NSLS), various tissues of the gall and leaf were scanned randomly. We scanned the parenchyma, mesophyll etc. Result showed that they have excess amount of Mn in both galls and scrub oak leaves [Figure 2 and 3].

### **Abandoned galls:**

It has been observed that Scrub oak sclerotize after active growth ceases. Exit hole is created when Cynipid wasp chews its way out, parasitoids chews its way out, Caterpillars chew its way in and birds damage the gall. Many but not all galls remain on bush through the next season. Ants, caterpillars and spiders were found in abandoned galls, and they have been identified to the family level. The spider and ants were identified as Dictyidae and *Leptothorax spinosae*, respectively. Results also showed that the more spider webs in galls, the less critters present [Figure 4].

### **Allegheny mound building ants:**

The mortality rate of *Petunias* planted on the ant mound (AM) and zone of death (ZD) is 100%. Ants aggressively attack the petunias [ Figure 5 for before and after]. They bite the plants and secrete formic acid, they also work together as a group, and each ant takes a small bite and leave. Allegheny ants do not attack Bear Berry and Pennsylvania sedge on their mound. They attack Pitch pine, Scrub oak and Huckleberry.

## **DISCUSSION AND CONCLUSION**

### **Galls:**

A primary cause of gall maker's mortality in field population is parasitism. Galls cannot grow for a long time because they are exposed to parasitoids with a mortality rate of 95.51%. Eulophidae, Torymidae and Perilamphidae were the three parasitoids we identified. The ovipositor of the parasitoid must be greater than the distance from the outer wall to the larva in order to deposit its egg on the cynipid larva. But, when the galls are large there is a thick parenchyma layer and a capsule separating them from the parasitoid, yet a Torymidae might be able to lay its egg on the cynipid larva due to its long ovipositor and cause the death of the cynipid larva.

Manganese (Mn) is essential for many plants to function. It helps in the assimilation of CO<sub>2</sub> in photosynthesis and aids synthesis of chlorophyll assimilation. High soil pH reduces Mn availability and low soil pH increases its availability even to the point of toxicity. Acid loving plants such as Blueberry, Pitch pine and Scrub oak tree may accumulate very high levels of Mn in their tissues due to the low soil pH [9].

Results from the National Synchrotron Light Source (NSLS) showed high Mn concentration in gall and leaf's tissue. This Mn comes from the soil and it might have accumulated in various tissues of the scrub oak plant in excess. This might be one of the things attracting the wasp to lay its egg on it. Also, previous studies showed that leaf has high chlorophyll concentration than gall. The gall has a spindle shape; it has no palisade and no stomata. These two things are important for photosynthesis to be effective. Therefore, we can say that gall is a modified leaf and it does not require all the essential elements needed by a regular leaf to survive.

**Abandoned galls:**

Once the wasp emerges, the abandoned gall seems like an attractive refuge for many arthropods. Woodier galls house arthropod community such as spiders, beetle and ants. Abandoned galls are old and sclerified. Sixty one percent of the galls we collected from the field have evidence of other non gall-maker's inhabitation. The two most common groups were spiders, *Dyctinidae* and ants *Leptothorax spinosae*. Approximately, 13.2% of the abandoned galls had spiders. *Dyctinidae* use the abandoned gall as a refugia because they are ambush hunters, so the abandoned galls makes an excellent hiding place for them to wait for prey [10].

On the other hand, the ant colonies accounted for 5% of the total numbers of abandoned galls with organisms. These ants might have colonized the galls because it provides them with a safe and comfortable place to breed. Therefore, many other organisms use these abandoned galls as temporary habitats.

**Allegheny mound building ants:**

Ants aggressively defend their mounds from any disturbance. They attack and kill some plants including scrub oak plants and *Petunias*. Due to the high frequency of Allegheny mound building ants in Pine Barrens forest, there would be a possibility to lose scrub oaks and eventually the cynipid wasp.

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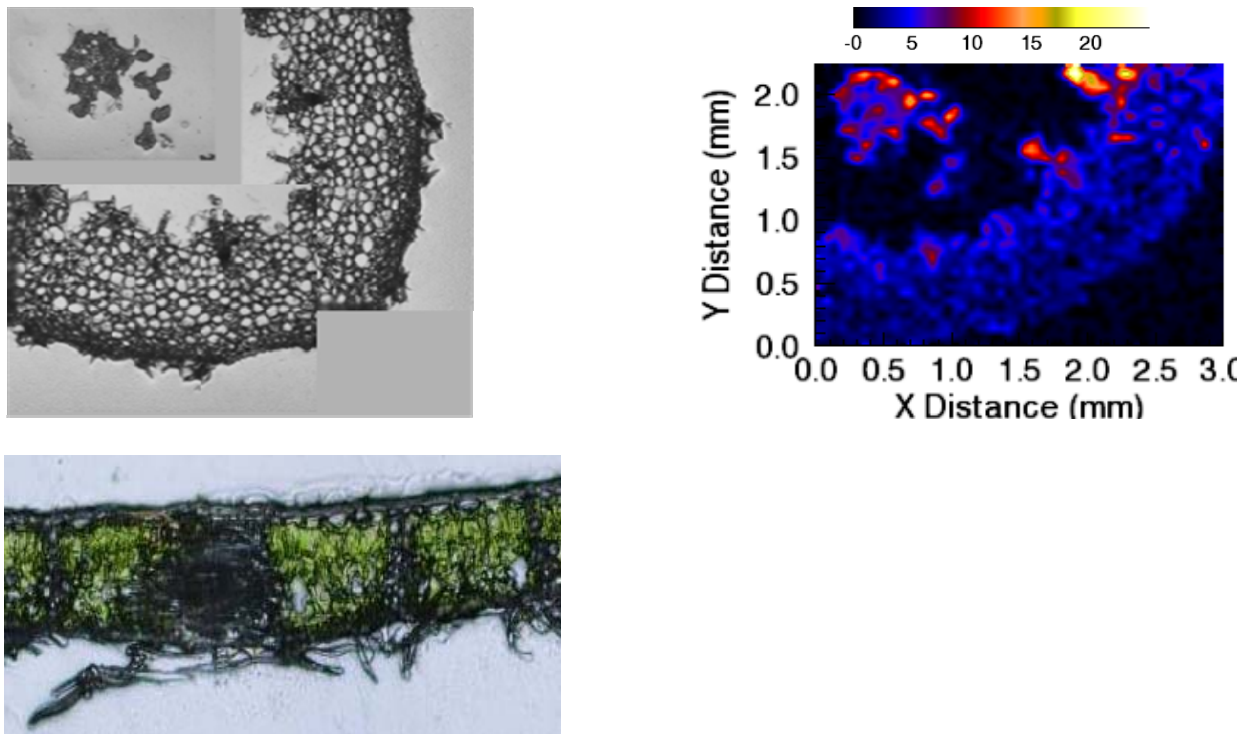
## Tables and Figures

### Figure (1)

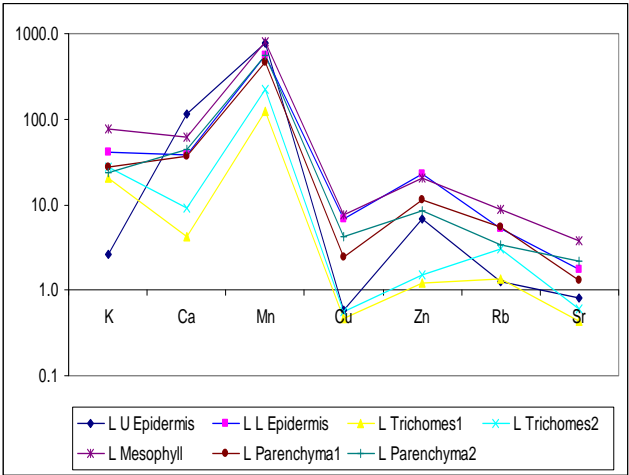
(a) Torymidae (b) Eulophidae and (c) Perilamphidae



Figure (2) a. Cryomicrotome Cross Sections of Cynipid Wasp Gall (30 $\mu$ m) and its histology, (b) XRF Image for Mn, c. Cross section of Scrub Oak gall (a and b) and leaf (c). (30 $\mu$ m)



**Figure (3)** Elemental concentrations in scrub Oak Leaf



**Figure (4)**

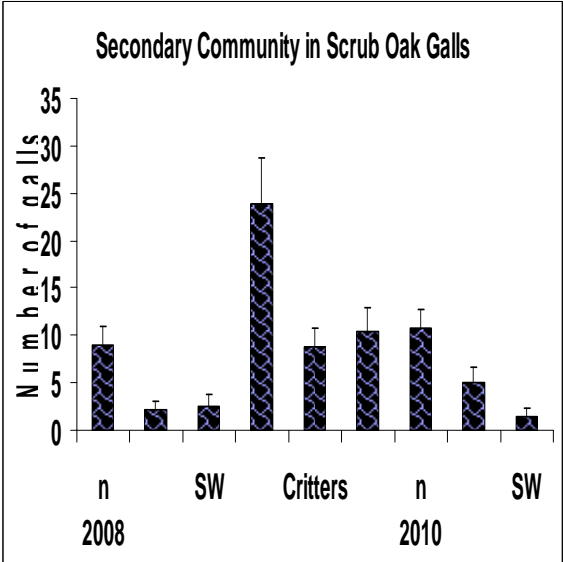
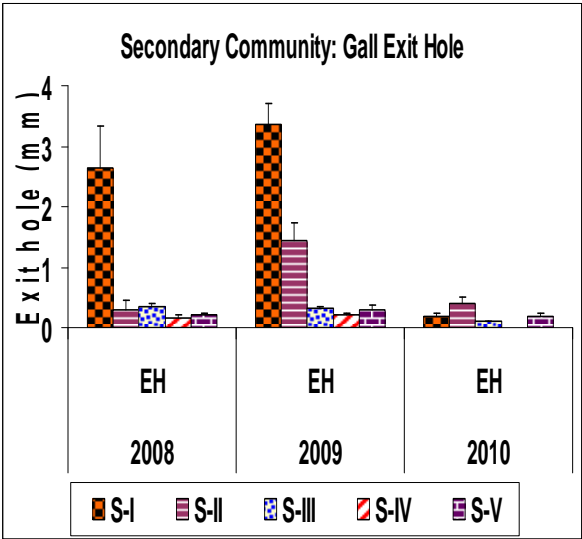




Figure (5) petunia on day 1 and Petunia on day 4