# Opportunistic Defense of the Bracken Fern, *Pteridium aquilinum*, by Patrolling Ants (Hymenoptera: Formicidae)

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Pierce Cedar Creek Institute URGE 2010

**Abstract**

In this study we established that a number of ant species defend bracken fern (*Pteridium aquilinum*) during the sensitive crozier stage by removing herbivorous insects from rapidly expanding fronds. Based on this premise, we further hypothesized that, at the end of the growing season, treated fronds from which ants were excluded would show a statistically greater amount of chewing-damage herbivory (total biomass lost) compared to untreated fronds from which ants were not excluded. Although our results show that ants do in fact remove some potentially harmful herbivores from bracken fronds during the crozier stage, statistical analyses comparing damage between treated and untreated fronds at the end of the growing season indicate that the amount of biomass lost to this type of herbivory is not significantly different.

Key Words: *Pteridium aquilinum*, bracken, fern, ants, nectaries, herbivory, opportunistic defense

## Introduction

The arthropod communities associated with ferns, especially bracken fern (*Pteridium aquilinum*), are of scientific interest because arthropods have failed to take full advantage of this widely available food source (Tempel 1981). Cooper-Driver (1978) estimates that only 9,300 insect species may use ferns as a food source, compared to approximately 400,000 species of insects that use angiosperms. Under-utilization may stem from the secondary plant compounds that protect the ferns from herbivorous arthropods (Cooper-Driver et al., 1977). Despite these chemical defenses, insect miners, gall formers, and borers avoid these toxins by utilizing non-toxic tissues of the phloem. Previous studies also show that bracken has developed a notable relationship with localized ant species in a variety of areas of its cosmopolitan distribution (Rashbrook et al. 1992).

Ants have been known to use the secretions derived from the axillary nectaries (AN) of bracken fern, often "patrolling" the plants and removing herbivorous species of potential harm to the fern (Bentley 1977, Buckley 1982, Douglas 1983, Beattie 1985). [Note: Axillary nectaries (AN) also have been called extra-floral nectaries (EFN) in the scientific literature (Chamberlain and Holland 2008).] A sample of Californian bracken has shown that nectary secretions include glucose, fructose, sucrose, maltose, and a variety of free amino acids (Douglas 1983). The idea that ants may be taking ownership of this fuel source is contentious, but not all scientists dismiss the idea (Cooper-Driver 1990). Bronstein et al. (2006) states, "…interactions between ants and EFN-bearing plants are often mutualistic, as EFN is a food resource that attracts and rewards ants that in turn protect plants from herbivory."

Why would bracken ferns continue to produce complicated, morphologically distinct nectaries that secrete complex mixtures of amino acids and sugars if not for some benefit to the plant? Indeed, several studies suggest that ants do not reduce the rate of herbivory of bracken (Tempel 1983, Heads and Lawton 1984, Heads 1986). Ness et al. (2009) proposes that these studies did not establish whether or not interaction of ants attacking a plant's natural enemies could translate into greater plant fitness, in the evolutionary sense. One study by Heads (1986) found that some aggressive ants of several species (e.g., *Camponotus* spp., *Formica* spp.) did remove non-adapted herbivores (those herbivores that do not feed on ferns), when they were experimentally introduced to the ants patrolling the bracken. This would suggest that even a small selective advantage for the fern would be a plausible evolutionary reason to retain the nectaries. For this reason, we hypothesized that bracken-ant symbiosis represents an opportunistic, mutualistic relationship that provides the fern with an active defense system when patrolling ants remove potentially harmful herbivores during the crozier stage--when pinnae are expanding and are most susceptible to damage. We sought to test this hypothesis by comparing the chewing-damage biomass lost over the growing season for experimental fronds that had ant access restricted and for control fronds where ant access was not restricted.

In addition to this study, we conducted a preliminary search for bracken fern in Peru, South America. Bracken fern has never been studied in Peru and has only been documented and observed in two other South American countries: Brazil (Martins et al. 1995) and Venezuela (Avila-Nunez et al. 2008).

## Methods and Study Sites

During the research period (April $15^{th}$ -August $15^{th)}$ 2010, we conducted experiments designed to compare the amount of chewing damage herbivory experienced by bracken fronds that were allowed to have ants regularly visit the nectaries, and those fronds to which we restricted ant access. We randomly selected experimental croziers, which we treated with Tanglefoot (Grand Rapids, MI), and control fronds which we left untreated. We took pictures of the ferns weekly from the crozier stage through senescence. These pictures were used to calculate the area of biomass loss due to herbivory over the term of the project.

The two sites (Plots 1 and 2) for our experiment were established within the property limits of the Pierce Cedar Creek Institute (PCCI), located in Hastings, Barry County, Michigan. The PCCI comprises 660 acres of protected land with diverse habitats (for complete information, see: *www.cedarcreekinstitute.org*). We began our experiments according to when the bracken croziers emerged within each site, which resulted in two experimental plots being established at slightly different times).

Plots 1 and 2 were located adjacent to each other with the White Trail passing between them. (Note: Plots 1 and 2 actually may represent a single plant, given the growth pattern of bracken fern's underground rhizomes, which could easily pass under the trail. For this reason we will use the word "frond" throughout this paper to identify the separate tripartite leaves of bracken that may possibly belong to only one individual plant.) Plot 1 was along the trail under thick forest canopy cover except for the margin of the plot near the trail on its west side; while Plot 2 was also along the trail but much more exposed without complete canopy cover. It was completely exposed to sun near the trail on its east side.

Plot 1 was set up with 20 treated experimental fronds and 20 untreated control fronds that were randomly selected. Plot 2 was set up with 10 experimental fronds and 10 control fronds. The total at the beginning of the project for experimental and control was 30 fronds each, for a total of 60 fronds.

The technique devised for the exclusion of ants was to cut clear drinking straws to a length of 12 cm and then slit the side of each straw along its entire length. We placed the straws on the raches (the vertical stems) of emerging croziers, with half designated as treated fronds and half as control fronds for each plot. The treated fronds had Tanglefoot (R) (an all natural substance used to restrict insect access to trees) applied to the outside of the straw to deter or entrap ants attempting to climb the raches and access the nectaries. Bracken fronds determined to be too near treated fronds were removed so that ants would not be able to bypass the Tanglefoot treatment by accessing the experimental frond from a neighboring frond. The untreated fronds also had straws placed over the raches, but the straws were not treated with Tanglefoot. All fronds, treated and untreated, were tagged with an identifying number.

Pictures were taken with a Cannon Rebel XS digital camera, approximately 1 meter away from where the rachis met the ground. A white background was used to ensure that only frond vegetation was calculated when analyzing area. Pictures were taken weekly, beginning with the week the plots were set up to document the expansion of each frond as well as the damage to each frond. Digital photographs were input into Photoshop to measure and compare the weekly biomass loss (in pixels) due to chewing damage. These data, collected over a period of 6 weeks, were analyzed statistically for fronds in Plots 1 and 2 separately. Procedures for photography of the fronds were similar to methods used by Tackenberg (2007) for biomass measurements of grasses; and photoshop methods were similar to those used by Lehnert (2010) for estimating butterflies' loss of wing area due to avian beak damage. Pictures were also taken to document ant visitors and arthropod interactions on the fronds, especially at the nectaries.

We conducted bimonthly samplings of arthropods associated with bracken. Arthropods were keyed out to the lowest taxon possible. (Note: generic keys were not available for some of the immature arthropods collected.) Pictures were also taken of non-adapted arthropods

(arthropods that are not known to feed in any life stage on bracken) when they were encountered on the bracken, but only those arthropods actually found imbibing at the nectaries were identified.

Fronds perished from a variety of factors ranging from frost, to deer foraging and human trampling, to frond lodging caused by boring insects. Those fronds that survived the duration of the study have been analyzed for chewing damage herbivory through comparisons of biomass amounts on each day pictures were taken. Pictures of chewing damage were not analyzed between June 5$^{th}$ and June 28$^{th}$ because the pictures were not taken using the same scale as the other pictures, making comparisons impossible.

In addition to diurnal observations of arthropod activity, nocturnal observations (until 11:00 pm) were also made to determine if arthropods visited the plants at night. These observations were only an initial attempt to determine how long ants were patrolling bracken and to determine if any other nocturnal arthropods were present.

Ant defense was tested by experimentally introducing non-adapted herbivorous insects (unidentified geometrid larvae collected from over-hanging trees or leaf litter within the plot area) to the fronds where ants were attending the nectaries. The larvae were added to the top of pinnae and allowed to freely crawl on the frond. We conducted 10 trials throughout the study period and recorded the reaction of the ants.

We performed an ANOVA using the Statistical Package for the Social Sciences (SPSS) to compare the amount of biomass destruction (at the end of the growing season) between the experimental plants (ants excluded by Tanglefoot) and control plants (ants not excluded).

**Results**

Plot 1 lost a greater proportion of fronds than Plot 2; however, both plots ended with similar surviving numbers of fronds from which we could compare biomass values. The average starting and ending biomass values for Plots 1 and 2 are quite similar, but Plot 1 lost more total biomass than Plot 2: 22% total loss for Plot 1 and 12% total loss for Plot 2 (Chart 1).

| | **Plot 1** | | | **Plot 2** | | |
|---|---|---|---|---|---|---|
| | Treated | Untreated | Total | Treated | Untreated | Total |
| Start | 20 | 20 | 40 | 10 | 10 | 20 |
| Died | 13 | 15 | 28 | 3 | 6 | 9 |
| Survived | 7 | 5 | 12 | 7 | 4 | 11 |
| Average Starting Biomass | 1,179,884 | 981,698 | 2,161,582 | 928,907 | 1,053,405 | 1,982,312 |
| Average Ending Biomass | 970,583 | 708,094 | 1,678,677 | 839,760 | 903,719 | 1,743,479 |
| Average Difference | 209,301 | 273,604 | 482,905 | 89,147 | 149,686 | 238,833 |
| Percent Loss | 18% | 28% | 22% | 10% | 14% | 12% |

Chart 1. The data accumulated for Plot 1 and Plot 2 from June 5$^{th}$-July 24$^{th}$.

In Plot 1 the untreated fronds started with less biomass than the treated; both remained close to the starting biomass values until the end of July when senescence began (Figure 1). In Plot 2 the treated fronds started with less biomass than the untreated, although the starting values for these are closer than those in Plot 1. While the biomass value for treated fronds stayed close to the starting value, untreated fronds experienced additional growth towards the end of June and then a drop in biomass in the beginning of July. Treated and untreated fronds in Plot 2 entered senescence with much closer biomass values than Plot 1 (Figure 2).

A comparison of only the treated fronds in Plot 1 and Plot 2 shows that they differed in the amount of original biomass. Also Plot 1 fronds experienced some growth in mid-July before beginning senescence at the end of July. Treated fronds of Plot 2 remained stable near the starting biomass value until the end of July when they began to senesce (Figure 3).

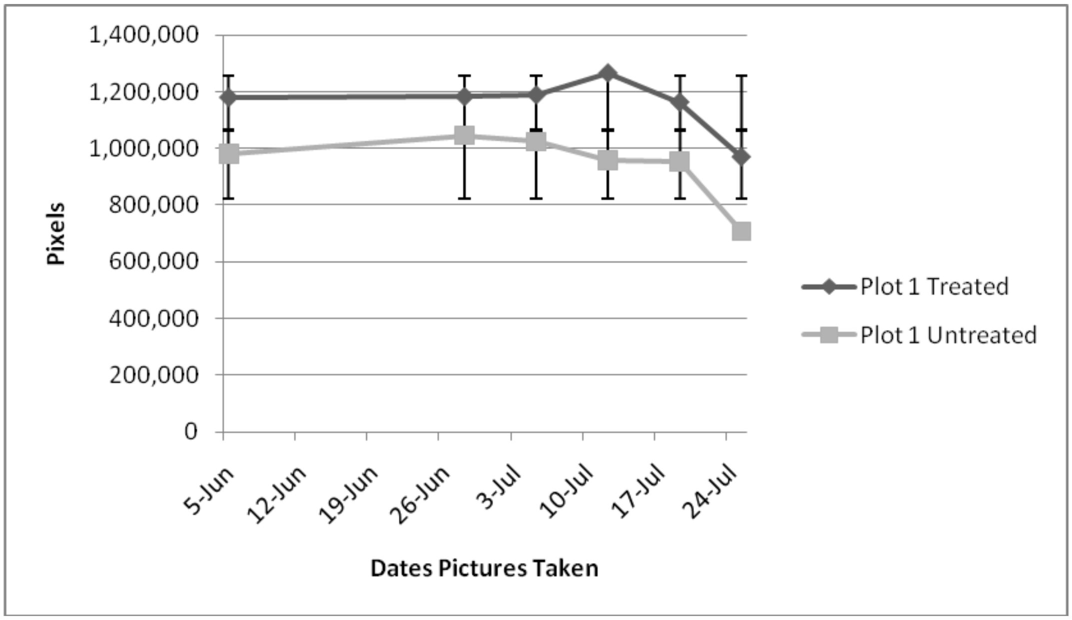


Figure 1. Average biomass values for treated and untreated fronds in Plot 1 for each date pictures were taken (showing error bars with 1 standard deviation).

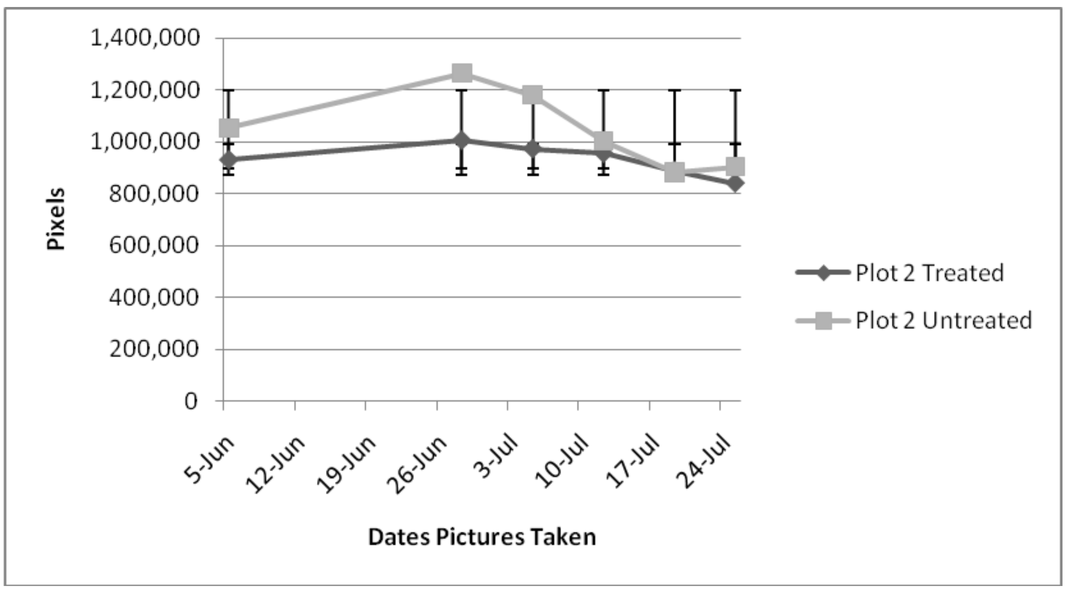


Figure 2. Average biomass values for treated and untreated fronds in Plot 2 for each date pictures were taken (showing error bars with 1 standard deviation).

A comparison of only the untreated fronds in Plots 1 and 2 shows that biomass values at the beginning of the study are very similar. Plot 2 untreated fronds experienced more growth in June and then more biomass loss in early July than Plot 1. Plot 1 overall biomass loss at the end of the study was less than that of Plot 2 (Figure 4).

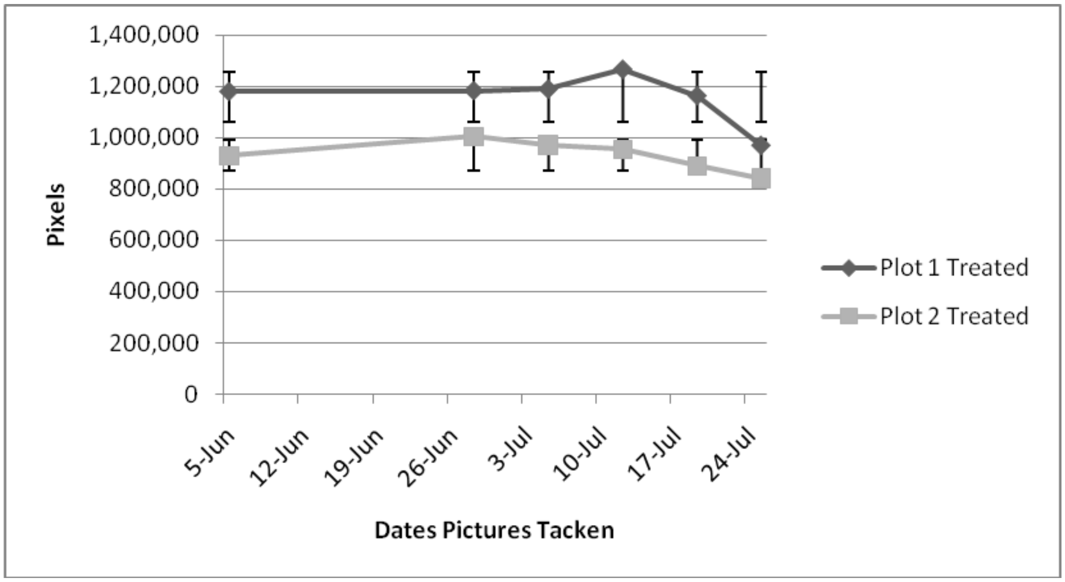


Figure 3. Average biomass values for Plots 1 and 2 treated fronds (showing error bars with 1 standard deviation).

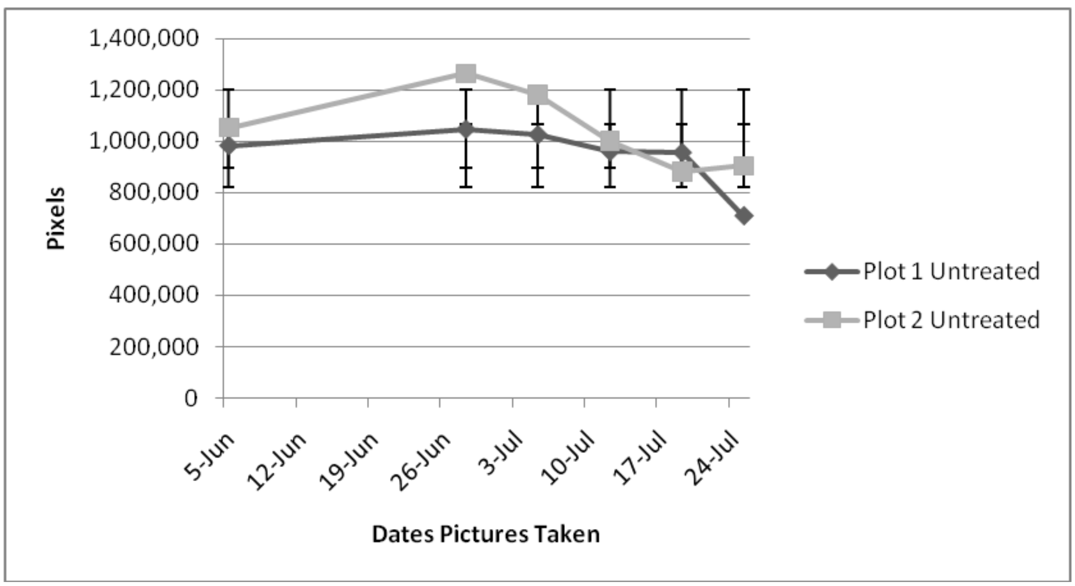


Figure 4. Average biomass values for Plots 1 and 2 untreated fronds (showing error bars with 1 standard deviation).

Beginning the study the biomass values for Plot 1 and 2, treated and untreated fronds, range between approximately 300,000 pixels; upon the beginning of senescence, after biomass loss over the study, the values are still within a range of 300,000 pixels. Even with different biomass losses for the treated and untreated for each plot, fronds stayed within the same range of values that they started (Figure 5).

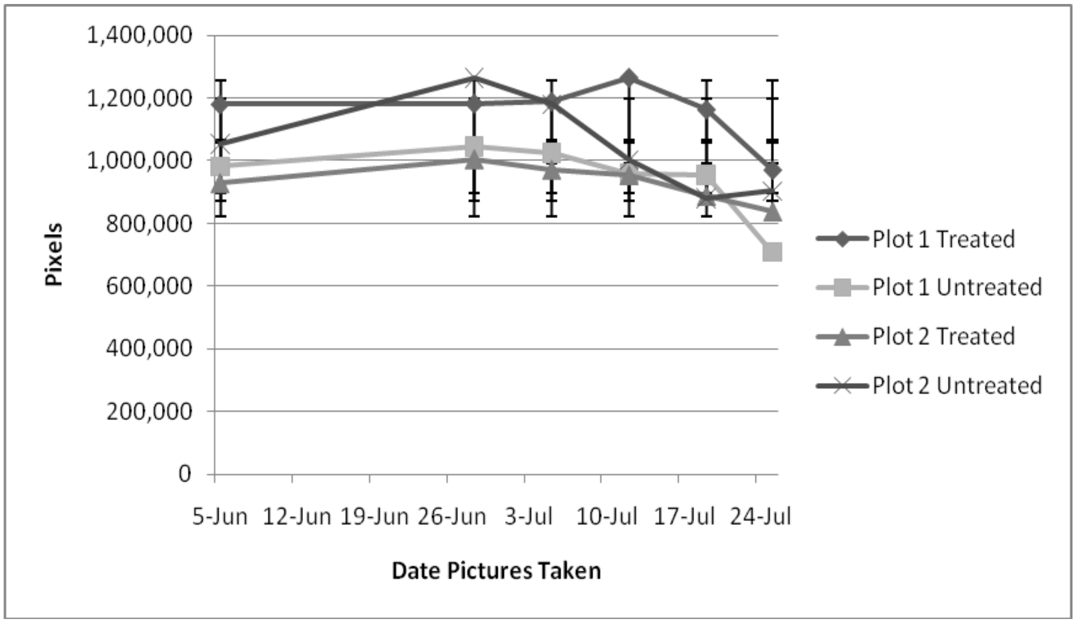


Figure 5. Averages of treated and untreated for both Plots 1 and 2 (showing error bars with 1 standard deviation).

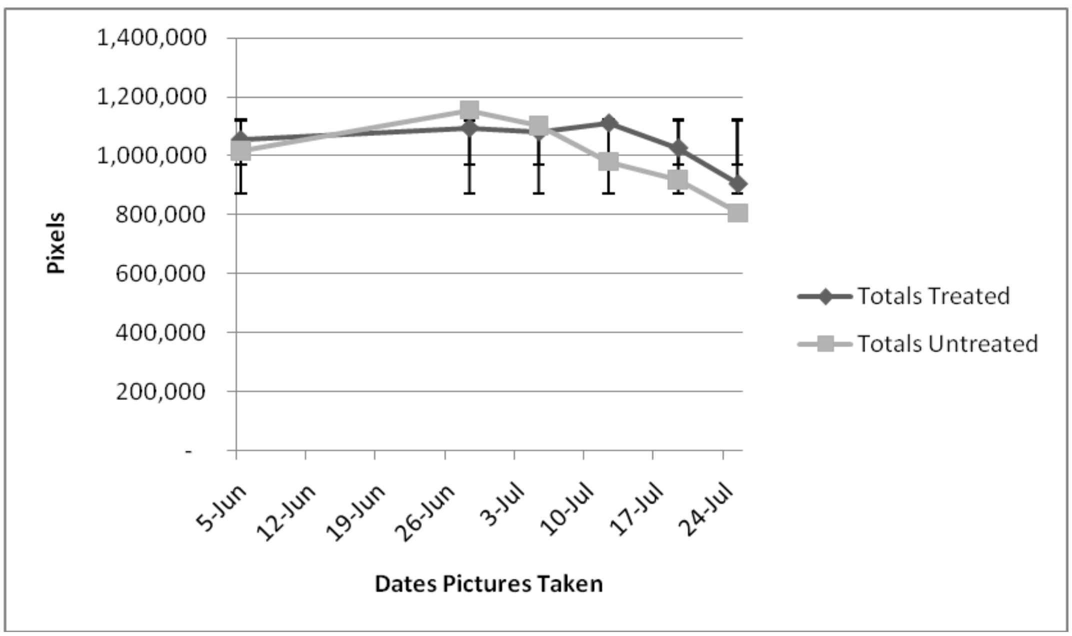


Figure 6. Averages for treated and untreated fronds when Plots 1 and 2 are grouped together (showing error bars with 1 standard deviation).

To see the general trend between treated fronds and untreated fronds we grouped Plots 1 and 2 together. The averages show that overall treated fronds and untreated fronds began with almost the same average biomass (total treated fronds had only 40,000 pixels more than total untreated). During the study the total untreated fronds had more biomass in the beginning of July than the total treated, but rapidly lost biomass over the remainder of July. When senescence began the total untreated fronds had lost more biomass than the total treated fronds (Figure 6).

Statistical analysis using ANOVA provided us with values $F_{1,20} = 0.244$ and $P = 0.626$. These results show that there was not a significant difference in the amount of biomass lost between the treated fronds (with Tanglefoot) and untreated fronds (controls without Tanglefoot).

**Arthropods Associated with Bracken**

Arthropods associated with bracken fern at PCCI during the study period were diverse but not abundant. Those transient non-adapted species not known to have phytophagous associations with the fronds (those consuming the leaf material at any stage in their life cycle) comprised members from 9 orders of insects and at least 5 families of spiders, Order Araneae. Other arachnids included at least several species of oribatid mites that make destructive bracken galls.

Also found in Plot 1 were associated (but non-adapted arthropods of bracken fern) in the following orders: Orthoptera, Dermaptera, Odonata, Hemiptera, Coleoptera, Neuroptera, Hymenoptera, Lepidoptera, Diptera. Phytophagous insects on bracken included pentatomid immatures and lycid beetles at nectaries.

For Plot 2 associated but non-phytophagous arthropods of bracken included: Orthoptera, Hemiptera, Hymenoptera, Lepidoptera, and Diptera. Phytophagous insects consist of numerous mite galls observed as well as pentatomid immatures at nectaries.

**Ant Defense**

A number of ant species visited bracken AN and transiently patrolled the fronds during the course of this study (Chart 2). Relative species diversity within Plot 1 was twice as great as that of Plot 2, despite the fact that these two plots were separated by less than two meters (the White Trail) along the plot lengths. The greater diversity of microhabitats within Plot 1 may have provided the base for this greater species diversity, but knowledge on this aspect of the study is inconclusive.

| **Hymenoptera (Formicidae)** | **Plot 1** | **Plot 2** |
|---|---|---|
| *Camponotus noveboracensis* | x | |
| *Camponotus pennsylvanicus* | x | x |
| *Formica aserva* | x | x |
| *Formica castaneus* | x | x |
| *Lasius (Acanthomyops) claviger* | x | |
| *Leptothorax muscorum* | x | |
| *Myrmica americana* | x | |
| *Myrmica punctiventris* | x | x |

Chart 2. Hymenopterous species collected during the bi-monthly sampling. All species listed were "patrolling" fronds in Plots 1 and 2.

Herbivores were experimentally introduced to ants attending the nectaries of bracken to record ant defensive behavior. In 9 of 10 trials done throughout the study, patrolling ants attacked the test herbivore until it either was expelled from the crozier or was killed. This data clearly shows that the ants do perform defense behaviors when presented with non-adapted herbivorous insects. However, whether or not this behavior was carried out depended largely on whether or not the introduced herbivore crawled down towards where the ants were attending the nectaries. Ants did not seem to notice the intruding herbivores when they took several minutes to reach the area where the ants were nectaring. In contrast, when ants left the nectary to patrol the plant, the test herbivore was immediately attacked when encountered. There was but one instance in which the ants did not pursue removing the herbivore from the frond.

## Discussion

The early warm temperatures in the spring of 2010 allowed the bracken croziers to emerge within the frost window and as a result, many of the fronds in Plot 1 were killed by a late, severe frost. Plot 2 fared better since the croziers were just beginning to emerge, and apparently were more resistant to frost than rapidly expanding croziers.

Plots 1 and 2 were adjacent to each other but exhibited differences in the plant diversity and density of the plots. Plot 1 was on the southeast side of the trail and had considerable tree cover, which shaded the plot from the direct sun in the middle of the day. However, Plot 1 had low plant diversity and except for trees, bracken made up the majority of the vegetation within the plot. Plot 2 was northwest of the trail and was located in a more open area, but this area appeared to have higher plant diversity and density with many plants competing with the bracken fern for area. The plants in this plot did not exceed a height of one meter and therefore the bracken fronds were in direct sunlight for most of the day.

### Biomass Loss

When Plots 1 and 2 are combined the results show that the treated fronds appear to experience less chewing damage herbivory over the course of the project than the untreated fronds show (14% biomass loss for treated and 21% for untreated.) This may be due to the fact that when restricting ants' access to the bracken ferns by application of Tanglefoot, we may have also restricted some larvae (e.g., geometrid larvae) access to the fronds as well.

### Exploratory Research in Peru

During our exploratory search for *Pteridium aquilinum* in Peru (conducted June 8$^{th}$-21$^{st}$)

we found locations in the cloud forest in Peru between the altitudinal zones that would include both *Pteridium aquilinum* ssp. *caudatum* and *Pteridium aquilinum* ssp. *caudatum* var. *arachnoideum. Pteridium aquilinun* ssp. *caudatum* appears mainly on disturbed land within primary wet and cloud forests at an altitudes ranging from 600 to 2400 meters above sea level, whereas *Pteridium aquilinum* ssp. *caudatum* var. *arachnoideum* inhabits a range of 1500 to 3000 meters above sea level (Alonso-Amelot and Rodulfo-Baechler 1996). We compared the pictures we had of the species in question with figures in Alonso-Amelot and Rudulfo-Baechlers 1996 paper, which described the morphological differences between the two subspecies of bracken. This led us to hypothesize that we encountered both subspecies. Our pictures show fronds that meet the characteristics described by Alonso-Amelot and Rudulfo-Baechlers for *P.a. caudatum* (an attached lobule of the pinnules and an triangular shape to the ends of the pinnae with shorter more tough, waxy fronds) and that of *P.a. arachnoideum* (a separate lobule located between pinnules with even length pinnules creating and oblong shaped pinnae with taller more fragile fronds).

It was our intent to record the arthropods associated with any bracken fern we found. However we did not find a diverse arthropod community on any of the Peruvian fronds. This may be because it was winter there and fronds were already fully expanded, or perhaps because the main area of study was along the steep roadside cliffs in the cloud forest area (because bracken primarily inhabits areas of disturbance) and the plants were continually being covered in thick layers of dust from the mountain road.

**Conclusion**

Observations over the course of our study consistently showed that ants were abundant on fronds in the crozier stage. However, as the croziers expanded the ants eventually stopped attending the nectaries, and as a result, herbivores were free to attack the fronds if they could counter or avoid the inhibitory secondary plant compounds produced by the ferns. At this point, ants stopped visiting the nectaries and ceased to patrol because the nectaries ceased to produce "nectar" after the fronds fully expanded. The ants then began to treat the expanding pinnae as they would any other plant. In effect, there was no longer any benefit to the ants and organized "patrols" ceased.

The crozier stage is the most sensitive stage in bracken fern's development (Cooper-Driver 1990). This is when the fern has the most nutritional value for herbivorous arthropods because the levels of tannins and cyanogenic compounds have not begun to increase and other secondary compounds bracken fern is known to produce have yet to be manufactured. The nectar secreted by bracken fern is produced only in this critical developmental period. When the fern expands, the secondary compounds are produced, nectar production ceases, and (formerly) patrolling ants leave.

The data show that there is no statistically measurable difference in the amount of biomass loss over the course of the growing season for treated fronds versus untreated fronds. Even though the amounts of chewing-damage herbivory over the entire season do not show a significant difference, it is obvious that ants can defend a frond to some extent. This defense however, is only pertinent when the ants are present, which is during the crozier stage, when the fern is secreting nectar.

Our study compared damage caused by chewing herbivorous arthropods over the entire growing season. However to actually determine if ants truly have a defensive impact, if would be necessary to quantify their defensive effects during the crozier stage when the ants are naturally present. It would definitely be worthwhile to find a way to measure the effects of restricting ants at this critical stage, when even minor damage could kill the entire frond. Also an experimental design that restricts only ants without restricting all potential herbivores would be preferred in future investigations.

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