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Measuring the Effects of "Opportunistic Defense" of the Bracken Fern, (*Pteridium aquilinum*) by Patrolling Ants (Hymenoptera: Formicidae) at Pierce Cedar Creek Institute in South Central Michigan

Ricki E. Oldenkamp^{1, 2} and Matthew M. Douglas¹

Abstract

In this study we show that in South Central Michigan (Pierce Cedar Creek Institute) eight ant species patrol bracken fern (*Pteridium aquilinum*) during the sensitive crozier growth stage. At times these ants remove herbivorous insects from rapidly expanding fronds. A new method for analyzing herbivory of bracken fern is employed to measure chewing damage to the fronds. Our results show that ants do in fact remove some herbivores from bracken fronds during the crozier stage; however, statistical analyses comparing the amount of chewing damage between treated and untreated fronds at the end of the growing season show no statistical difference.

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 food source is contentious (Tempel 1983, Rashbrook et al. 1992), but not all biologists dismiss the idea (Cooper-Driver 1990). Bronstein et al. (2006) state, "...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."

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Why would bracken ferns produce complex, 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 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 herbivores that were experimentally introduced to bracken. A study by Koptur et al. (1998), involving other ferns with nectaries (*Polypodium* spp. of Mexico), has shown a significant difference in damage between ant-excluded fronds and control fronds. These studies suggest that even a small selective advantage for the fern would be a plausible evolutionary strategy for maintaining nectaries. For this reason, we hypothesized that bracken-ant mutualism represents a 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 area lost over the growing season for experimental fronds with ant access restricted and for control fronds where ant access was not restricted.

Methods and Study Sites

During the research period, 15 April - 15 August 2010, we conducted experiments designed to compare the amount of chewing damage 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. Ferns were photographed weekly from the crozier stage through senescence. These photographs were used to calculate the area of chewing-damage 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 when the bracken croziers emerged within each site, which resulted in the 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.

To exclude ants we cut clear drinking straws to a length of 12 cm and then slit the side of each straw along its entire length. The straws were placed around the rachis (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® (The Tanglefoot Co., Grand Rapids, MI) applied to the outside of the straw to deter or entrap ants attempting to climb the rachis and access the nectaries. Bracken fronds in close proximity to treated fronds were removed so that ants could not bypass the Tanglefoot® treatment by accessing the experimental frond from a neighboring frond. The untreated fronds also had straws placed over the rachis, but the straws were not treated with Tanglefoot®. All fronds, treated and untreated, were tagged with an identifying number.

Photographs were taken with a Canon Rebel XS digital camera at a distance of 1 meter from where the rachis met the ground. A white background was used to ensure that only frond vegetation was calculated when analyzing area. Photographs 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 Adobe Photoshop® to measure and compare the weekly area loss (in pixels) due to chewing damage. These data, collected over a period of six 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 Adobe Photoshop® methods were similar to those used by Lehnert (2010) for estimating butterflies' loss of wing area due to avian predators. Photographs also documented ant visitors and arthropod interactions on the fronds, especially at the nectaries.

In our bi-monthly sampling we documented, and recorded to the ordinal level, the transient non-adapted arthropods that were associated with bracken fern. We did not witness herbivory and because of this we were not able to document the actual arthropod herbivores.

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 were analyzed for chewing damage herbivory through comparisons of surface area amounts on each day photos were taken. Photographs of chewing damage were not analyzed between June 5 and June 28 because the photographs were not taken at the same scale, making comparisons impossible.

Ant defense was tested by experimentally introducing herbivorous arthropods (unidentified geometrid larvae collected from over-hanging trees or leaf litter within the plots) 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 chewing-damage destruction (i.e., loss of surface area due to chewing damage alone) at the end of the growing season between the experimental plants (ants excluded by Tanglefoot®) and control plants (ants not excluded).

Results

Plot 1 was established with a greater number of fronds than Plot 2, but also lost more fronds over the study resulting in a similar number of fronds being analyzed between the two plots. Overall in Plots 1 and 2 the treated fronds lost less surface area than the untreated fronds. When the losses for both plots are combined, the treated fronds lost 14% total surface area while untreated lost 21% (Table 1).

Figure 1 shows the total average values in pixels for treated and untreated fronds for each day photographs were analyzed over the study. Treated and untreated fronds both experienced growth with an increase in surface area;

Table 1. Number of fronds starting and ending the study, average surface area (pixels) per frond starting and ending the study, and the difference in surface area for fronds in Plot 1 and Plot 2 from June 5th-July 24th.

	Plot 1		Plot 2		Combined			
	Treated	Untreated	Total	Treated	Untreated	Total	Treated	Untreated
Start	20	20	40	10	10	20	30	30
Died	13	15	28	3	6	9	16	21
Survived	7	5	12	7	4	11	14	9
Average Starting Surface Area	1,179,884	981,698	2,161,582	928,907	1,053,405	1,982,312	2,108,791	2,035,103
Average Ending Surface Area	970,583	708,094	1,678,677	839,760	903,719	1,743,479	1,810,343	1,611,813
Average Difference	209,301	273,604	482,905	89,147	149,686	238,833	298,448	423,290
Percent Surface Area Loss	18%	28%	22%	10%	14%	12%	14%	21%

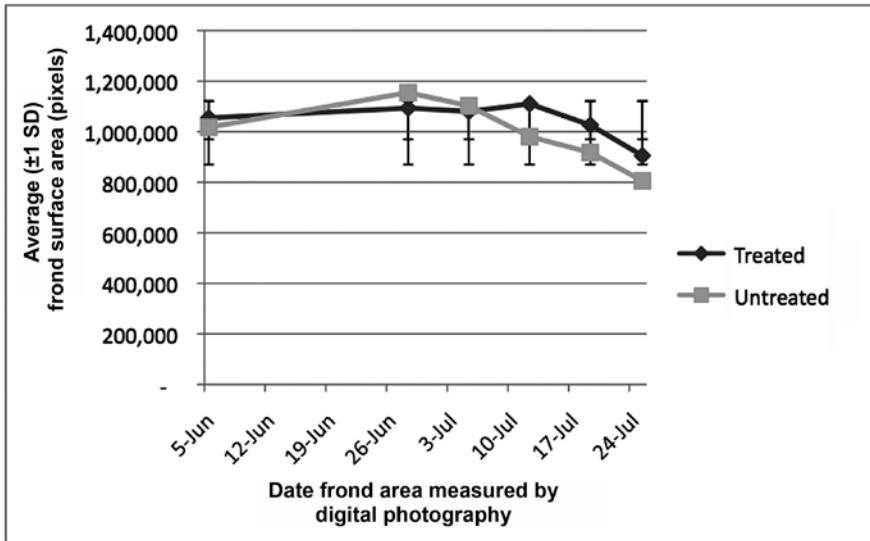


Figure 1. Average surface area (pixels) of treated and untreated fronds for Plots 1 and 2 combined (showing error bars with 1 standard deviation, $n = 30$ at the start)

Table 2. . Hymenopterous species collected during bi-monthly sampling. All species listed were "patrolling" fronds in Plots 1 and 2. The symbol x indicates the species was present in the corresponding plot.

Hymenoptera (Formicidae)	Plot 1	Plot 2
<i>Camponotus noveboracensis</i> (Fitch)	x	x
<i>Camponotus pennsylvanicus</i> (De Geer)	x	x
<i>Formica aserva</i> (Forel)	x	x
<i>Camponotus castaneus</i> (Latreille)	x	x
<i>Lasius (Acanthomyops) claviger</i> (Roger)	x	
<i>Leptothorax muscorum</i> (Nylander)	x	
<i>Myrmica Americana</i> (Weber)	x	
<i>Myrmica punctiventris</i> (Roger)	x	x

before the chewing-damage herbivory loss until senescence. The averages show that overall treated fronds and untreated fronds began with almost the same average surface area (total treated fronds had only 40,000 pixels more than total untreated). During the study the total treated fronds had less surface area in the beginning of July than the total untreated, but the untreated rapidly lost surface area over the remainder of July. When senescence began the total treated fronds had lost less surface area than the untreated fronds.

Statistical analysis using ANOVA show that in this study there was not a significant difference ($F_{1,20} = 0.244$; $P = 0.626$) in the amount of surface area 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. Measuring non-adapted arthropod species diversity at bracken fern was not a major focus of this study; however, 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 consisted of numerous mite galls as well as pentatomid immatures at nectaries.

Eight ant species visited bracken AN and transiently patrolled the fronds during the course of this study (Table 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.

Ant Defense. Herbivorous arthropods 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. 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.

Surface Area 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 study than the untreated fronds (14% surface area 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.

Ant Defense. In 9 out of the 10 trials where herbivores of bracken were experimentally introduced the ants evicted or killed the herbivores. This data clearly shows that the ants do perform defense behaviors when presented with herbivorous arthropods. However, whether or not this behavior was carried

out depended largely on whether or not the introduced herbivore crawled down toward the ants 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 attending to the nectaries. In contrast, when ants left the nectary to patrol the plant, the test herbivore was immediately attacked when encountered.

Conclusion

Our study consistently showed that ants were abundant on fronds in the crozier stage. However, as the croziers expanded ants stopped visiting the nectaries and ceased to patrol because the nectaries ceased to produce "nectar". The ants subsequently began to treat the expanding pinnae as they would any other plant. Herbivores were then free to attack the fronds if they could counter or avoid the inhibitory secondary plant compounds produced by the ferns.

The most sensitive stage in bracken fern's development is the crozier stage because even minor damage can stunt or kill the frond. The crozier stage is targeted because it is when the fern has the most nutritional value for herbivorous arthropods since 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 (Cooper-Driver 1990). The nectar secreted by bracken fern is produced only in this critical developmental period. As the fern expands, the secondary compounds are produced, nectar production ceases, and (formerly) patrolling ants leave (Douglas 1983, Cooper-Driver 1990).

The data show that there is no statistically measurable difference in the amount of surface area loss over the course of the growing season for treated fronds versus untreated fronds. Even though the amounts of chewing-damage herbivory at the end of the season do not show a significant difference, ants may be able to defend a frond to some extent. We witnessed ants evicting or killing herbivores when they were experimentally introduced. 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 determine if ants have a defensive impact, it would be necessary to quantify their defensive effects during the nectar-producing crozier stage when the ants are naturally present. It would 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 ants without restricting all potential herbivores would be preferred in future investigations.

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