THE PRESENCE AND FUNCTION OF NECTARIES ON THE BIG-TOOTHED ASPEN, POPULUS GRANDIDENTATA

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ABSTRACT

Whitman, Deborah L. The presence and function of nectaries on the big-toothed aspen, Populus grandidentata. The function of the nectaries was studied by obstructing the nectaries on the treated plants and comparing their percent herbivory of these plants and insect populations on them to that of control plants. Ants were found to be attracted to the control plants, and fewer herbivores were found on those plants also. The percent herbivory and absolute area of herbivory did not show significant differences between the control and test groups. Based on the implications of the insect data, it was hypothesized that artificiality and seasonality may have affected the results of the herbivory test. The hypothesis that ants are attracted by the nectaries to repel herbivores was not proven by the data but some evidence was presented to support it.

INTRODUCTION

The more obvious relationships between plants and animals tend to be parasitic relationships in favor of the animals. Animals consume plants, build their homes in plants, lay their eggs in plants, etc., usually to the detriment of the plant. Pollination is the most widely-observed exception to this trend, in that both the plant and the animal (usually) gain some benefit from the plant/pollinator relationship.

In this study, another system that is suspected of being mutualistic between insects and plants is explored. The plant, the big-tooth aspen, Populus grandidenta bears small glands at the base of the leaf where it joins the petiole. The contents of these glands are very effective insect attractants--especially for ants and small black flies. The formation and maintenance of these "nectaries" must involve some energy from the plant, and thus one might expect that if plants with nectaries have been selected for, perhaps the presence of nectaries confers some benefit on the plant. Initial observations of large numbers of ants at the nectaries suggests that perhaps the function of the nectaries is to attract ants which would then hypothetically "protect" the plant from herbivores. A similar relationship has been documented by D. Janzen in 1966 where acacia trees in Central America provide nesting sites and food for the ants, while the ants provide the tree with protection from insects and other plants.

A second possibility was suggested by initial observations as to the function of the nectaries. A rather large beetle, an insect that would normally be considered herbivorous, was observed feeding at the nectaries. This raised a question of whether or not the nectaries might be providing an alternative

food source to the herbivores, to decrease the loss of leaf area due to herbivory.

To test these two hypotheses I intended to obstruct the nectaries of some big tooth aspen shoots and compare their resulting degree of herbivory and their "on plant" insect populations to a control group of plants.

MATERIALS AND METHODS

My initial observations were made at a cutting site on University of Michigan Biological Station property, about 2 miles west south of camp. The area was scheduled to be burned in the midst of the experimental period, and this detracted from the attractiveness of the area as a study site.

A similar site was located right behind Lakeside Lab at the Station. It was just off Hilltop road to the right. The site had been cleared about a year earlier to create a location for the study of acid rain in our area, and was referred to as "The Acid Rain Site".

When the aspen tress in the site were cut down, the stumps remained rooted, and this year those roots sent up suckers. The site contains a large population of these suckers, which are continuing to put out new leaves this late into the season.

Two transects were taken perpendicular to one another to better cover more of the cleared area. Beginning at an arbitrarily chosen starting point, the first 16 plants on either side of the tape measure were chosen to make up the first transect. The same procedure was repeated for the second transect, but the number of plants used was 14, making the toal sample size 30.

Control and treated plants were alternated one after the other, with a flip of a coin determining that the first plant would be a control. Each plant was flagged with either yellow (control) or orange (treatment) flagging. Before any treatment was administered, the outlines of the top four leaves of each plant were traced, to determine the amount of herbivory (more fully discussed below).

After this, the treatment plants had their nectaries blocked up by dabbing a small drop of clear nail polish on them.

Presence of Insects

After the plants had been treated, three counts were taken (on three different days) of the number of insects found on the top four leaves of each plant during a two minute observation period. Three categories of insects were tabulated: 1)ants 2)herbivores 3)small black flies, and the number of these insects that spent time at the nectary site was noted also, to determine if the nectaries were indeed the attractive stimulus.

Amount of Herbivory

As mentioned earlier, tracings were made of the top four leaves of each plant before the treatment was administered. The actual outline seen in a tracing can be extrapolated to indicate what the outline of the leaf would have been had it not been herbivorized. This extrapolation process was done for each leaf (see Figure 1). The extrapolated area was then determined using the leaf area machine. The "herbivorized" segments were then removed from the tracing, and the actual remaining area was measured.

After a period of seven days following the treatment (during

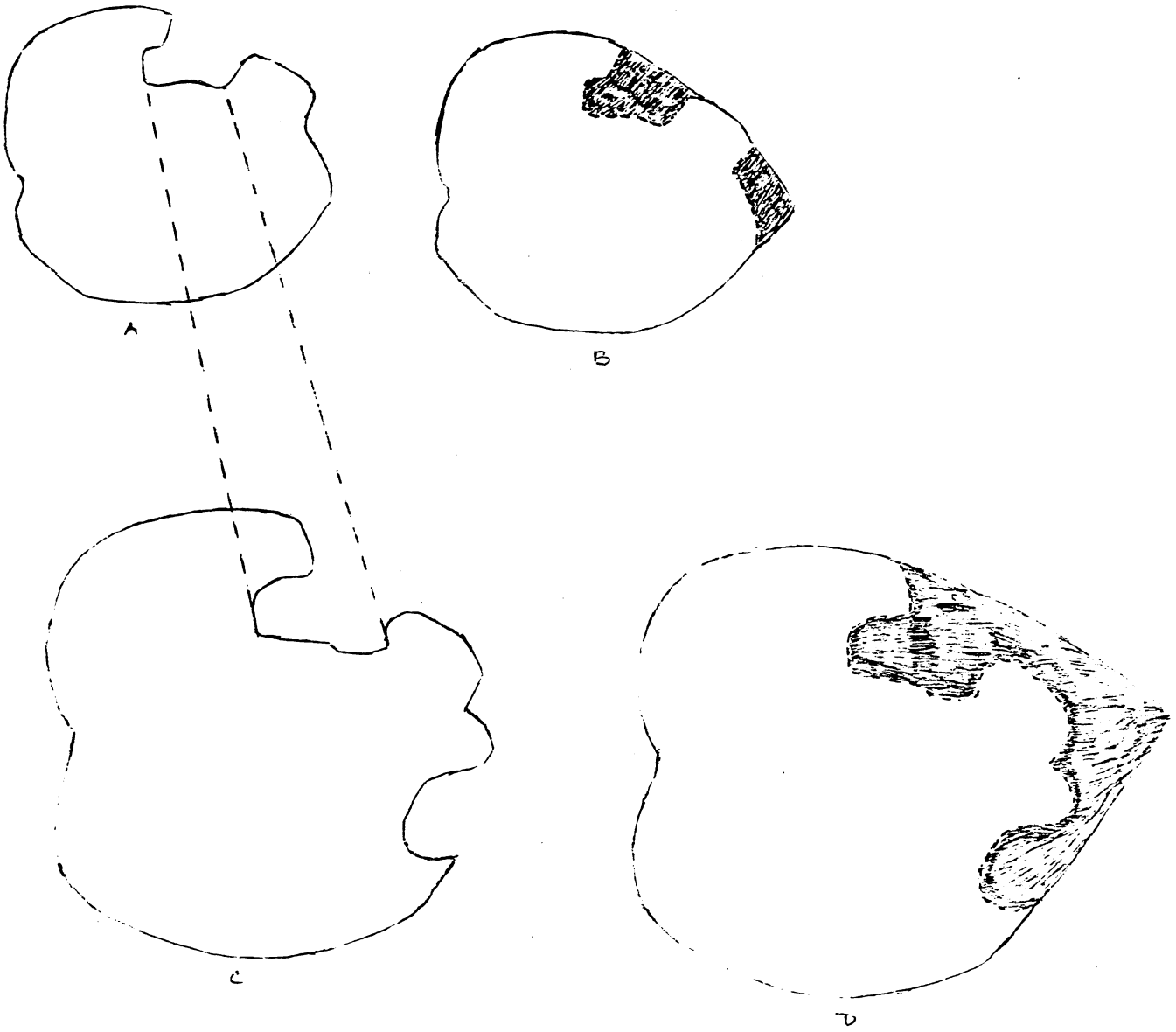

Figure 1. The extrapolation of leaf area before herbivory.
A. Herbivorized area on day 0. B. Extrapolated area on day 0
C. Herbivorized area on day 7 C. Extrapolated area on day 7.
Note the "growth" of the herbivorized area between A and C.

which time the plants were checked daily to ensure that the nectaries remained blocked), the leaves were again traced and the overall area extrapolated. The change in the percent herbivory (change in the percentage of leaf area eaten) and the change in the actual area gone to herbivores, were compared for the control and treatment groups.

RESULTS

Nectaries tend to be found on newer leaves. When a leaf first unfolds, it does not contain nectaries, but usually forms them within the first day. As one looks down the length of one of the suckers, leaves can be seen with differing stages of nectary developement. Smaller leaves usually have only two nectaries--one on each side of the petiole. Larger leaves will often have three to four nectaries, and I have observed leaves with as many as six nectaries clustered around the petiole. The nectaries often seem to increase slightly in size as the leaf does, usually getting no larger than a millimeter in diameter. It would appear that the nectaries atrophy as the leaf gets older, because some larger leaves can be seen with nectaries that are "dried up," shriveled and black in color. No insects are usually seen at nectaries in this state. Finally, one sometimes finds older leaves at the base of taller suckers which have no nectaries at all, although one may be able to see scars where the nectaries had been.

The nectaries ranged in color from light green to dark brown and later to black as they began to atrophy. Under a dissecting scope, they appear as small, irregular circles, with an outer ring of raised height, and an inner area that is sunken down.

Within the sunken area are smaller "bumps" sticking up, usually one to four per nectary. When pierced, these bumps are shown to contain liquid, presumably the nectar which attracts insects to the nectaries.

Insects observed eating at the nectaries employed two different strategies based on their morphology. The small black flies were observed inserting the proboscis into the nectary well. Ants, and the beetle observed, used their short mandibles and would stick first one, then the other side of the mandible into the well and move it around with a scraping type motion.

Presence of Insects

The results of the data on insect presence are tabulated in Table I, and briefly show that many more ants were found on the control plants (53) than on the treated plants (10). This is a significantly higher incidence of ant presence according to the Mann-Whitney-U test ($U=55$, tabular value=59, $p<.05$). It should be mentioned however, that the high number of ties found in the data makes the validity of using such a statistical test a little questionable. Numerically though, the numbers, 53 vs. 10 speak for themselves.

Of the 53 ants observed on the control plants, 46 of them (87%) were observed visiting the nectaries during the two minute observation periods. Only one ant was observed spending any time at the nectary site in the treatment plants, and it did not appear to be feeding.

The number of herbivore insects observed on the treated plants was 15, compared to 6 seen on the control plants. One of these insects, Graphocephalus (Cicadellidae), was seen at the nectary on a control plant, but I was unable to tell if it was feeding.

CONTROL PLANTS

PLANT #	8/7/80			8/9/80			8/10/80		
	A	H	BF	A	H	BF	A	H	BF
1	2	1	0	0	0	1	0	0	1
2	0	0	0	1	0	0	1	0	0
3	3	0	0	1	0	0	0	0	0
4	1	1	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0
7	3	0	0	5	0	0	5	0	1
8	0	0	1	0	0	0	1	0	0
9	0	0	0	0	1	0	0	0	0
10	0	0	0	0	0	1	0	0	0
11	6	0	0	8	0	0	6	0	0
12	0	0	0	1	0	0	0	0	0
13	1	2	5	2	0	0	0	0	0
14	0	0	0	0	0	0	1	0	0
15	0	0	1	2	0	0	2	1	0

TREATED PLANTS

PLANT #	8/7/80			8/9/80			8/10/80		
	A	H	BF	A	H	BF	A	H	BF
1	0	0	0	0	0	0	0	1	0
2	1	0	1	0	0	0	1	1	0
3	0	0	1	0	0	0	0	1	0
4	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
6	1	0	0	0	0	2	0	1	0
7	0	0	0	1	0	0	0	0	0
8	0	0	0	1	1	0	0	0	0
9	0	1	0	0	0	0	0	0	0
10	1	0	0	0	1	0	0	0	0
11	0	0	0	1	1	0	0	1	0
12	0	2	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0
14	0	3	0	1	0	0	0	1	0
15	0	1	0	2	0	0	0	0	0

TABLE I. Tabulated data for number of insects present on three different days. The observational periods were 3 minutes each. The symbols represent: A = ants, H = herbivorous insects, BF = black flies.

No herbivores were found to spend time at the nectary sites of the treatment plants.

The black flies were found more abundantly on the control plants than the treated plants. Twelve were observed on the control plants and three observed on the treated plants. Only four of the twelve flies on the control plants visited the nectaries during the two minute observation periods. One fly was seen at the nectary site on a treated plant, but the proboscis was not extended.

Amount of Herbivory

The change in the absolute area missing due to herbivory was calculated and the mean area eaten away from the control plants in the seven day period was 19.14 ± 28.34 cm^2. For the treated plants, the mean area eaten away was 8.97 ± 7.809 cm^2. However, when the two sets of values were compared by the Mann-Whitney-U test, no significant difference was found in the amount of area gone to herbivores in the control and treated plants ($U=73$, tabular value=59, $p>.05$).

The change over one week in the percent herbivory was also calculated, and the mean value for the control plants was 7.52 ± 7.44, and for the treated plants was 3.70 ± 2.84. Again, although the means are different, no significant difference was found between the percent herbivory for the control and treated plants as determined by the Mann-Whitney-U test ($U=84$, tabular value=59, $p>.05$).

DISCUSSION

The prevelance of nectaries on the younger leaves suggests

that if the nectaries do function to protect the plant from herbivory, that their main purpose is to protect the leaf when it is young and tender. The older leaves have a more leathery covering and potentially a higher concentration of secondary compounds that might help to reduce herbivory of their surface area. Thus, the nectaries may be a trait that has been selected for to decrease the loss of surface area in young leaves. The very young leaves when they unfold, are very fuzzy, which probably also protects them from herbivores. Then as they form nectaries, the leaf gradually loses its hairs, especially on the upper surface. If the nectaries do function in decreasing herbivory, they probably work in conjunction with the hairy surface area to produce this effect.

It should be noted that these observations were the impetus behind choosing the top four leaves on every plant to conduct this study. Presumably the effects of having nectaries would be most prominent on the top (youngest) leaves.

Insect Presence

There did appear to be significantly more ants found on the plants with functioning nectaries. Remembering also that 87% of the ants visited the nectaries during the observation period, this would lend support to the hypothesis that the nectary's function may be to attract ants.

It can also be noted that fewer herbivores were found on the control plants. Only one herbivore was found on any plant that had more than one ant present during any given observation period. This data is based on very few samples, but it does suggest that the ants may be repelling the herbivores. In my observations, I never saw an ant attack an herbivore,

but I also never saw an herbivore approach a leaf where an ant was present. This might suggest that the actual presence of the ants is sufficient to keep herbivores away. The plants without functioning nactaries did not seem to attract as many ants, and they did have a higher number of herbivores than the controls.Although it is not possible to conclusively say from my data that the former is the cause of the latter, the data is suggestive on this point.

The data does not seem to support the hypothesis that the nectaries provide an alternative food source to the herbivores. Only one herbivore was seen at the nectaries and it was not clear whether or not it was feeding. This is not a high enough incidence of this type of phenomena to support this hypothesis.

The high abundance of black flies on the control plants was expected from my initial observations. What was surprising however, was that only four of the twelve flies were observed visiting the nectaries. It is possible that the two minute observation period was not long enough to account for the true rate at which they visit the nectaries. Also,the fact that fewer black flies were found on the plants without nectaries suggests that perhaps the nectaries are the attractive force drawing in the black flies. I made no hypothesis about the potential benefits these insects might confer upon the plant. They seem to be attracted to the nectaries as the ants are, but I am unable to see how they could be of much use to the aspens. It is doubtful that they protect the plant from herbivory due to their small size. It is unclear whether or not

their presence actually hurts the plant since they do not seem to be herbivorizing the leaves. Thus, one might suggest that thier presence at the nectaries is not an "intended" function of the nectaries, but they are "inadvertantly" attracted to the nectaries where they feed without conferringany benifit to the plant. If this is so, it could be said that they are possibly hurting the plant to some degree, by "stealing" nectar but providing no benifits.

Amount of Herbivory

Based on the insect presence data, it seems that the nectaries do attract the ants, and that the presence of those ants is probably reducing the number of herbivores on a plant. Thus, one would expect the amount of herbivory to be less for the control plants than for the test plants. However, both the percentage of herbivory and the absolute area of herbivory data do not lend support to this hypothesis. In fact, based on the means, the control plants actually lost more area and a higher percentage of their leaf area during the seven day experiment. However, the very high standard deviations for this data make me very hesitant in using those mean values. Instead, one should look to the statistical test, and according to those results there is no significant difference between the control and treated plants. This data suggest that the nectaries have conferred no benefits based on amount of herbivory to the plants that bear them.

The insect data suggests that the initial stages of the mutualistic relationship are functioning--the plants are attracting ants to the nectaries, and to at least some extent,

the ants (while they are on the plant) seem to be keeping the herbivore numbers down. But somehow the final benefit is not being incurred on the plants with the nectaries. Several possible explanations occur to me that may account for this, and more likely than not, many of these factors discussed below may be working in conjunction here.

First of all, the site itself has been altered drastically, and represents an environment that would only rarely (perhaps after a fire) be encountered in nature. The suckers themselves are not usually sent up in such abundance and close proximity. This may drastically alter the relationship as it would normally exist in nature. In the clearing the ants have many plants in close proximity and they may be gathering nectar from all of them, but not spending enough time at each plant to confer any "protection" on it. The data on insect presence suggest this. Most plants had ants during one or two of the observation periods, but only two plants, #7 Yellow and #11 Yellow, had ants present on all three days. These two plants were fairly isolated within the site with respect to the other control plants, while most of the other plants tended to be clumped together. This may have made it more beneficial for ants on these two plants to remain there, instead of moving to other plants located relatively far away. Plant #7 Yellow had a very low percentage of herbivory (in fact it actually lost so little to herbivores and grew so much that the percentage gone to herbivores was less after one week than initially). This might suggest that the ants were conferring a benefit on this plant. However, plant #11 Yellow did not show similar

results, even though its ant population was also consistantly high. This may be due to chance effects, or it could be due to a combination of the other factors considered below. Also, it should be noted that the overall abundance of ants (and black flies) was much lower at this site than at the first site I spent some time at. This further suggests that if the ants had needed to spend more time at any one plant, they might have provided a greater benefit to the plants.

Secondly, it is possible that the nectaries are intended to carry out their prime function in the spring, when the plant is more likely to be putting out new leaves, and the abundance of herbivores is much higher. Many herbivorous insects are larval forms and transform during the summer. Caterpillars are the most obvious example, and indeed, only one caterpillar was found during my observations. It may be that the conditions this late in the season are not those at which the nectary's optimal functionality is expressed.

Finally, the length of time over which the experiment was conducted may not have been sufficient to allow for the expression of any benefits the nectaries may provide. From looking at the leaf tracings (and as seen in the data) some "new" herbivory certainly took place over the seven day period. However, it is possible that a longer period of time would be needed to show significant differences between the two groups. This could be especially true if looked at in conjunction with the seasonality consideration mentioned above. Earlier in the season, the plant probably encountered more herbivorous insects and suffered more herbivory in a shorter period of time. During this time of year, it may be that the effects of herbivores are so small that the nectaries aren't conferring the strong

benefit they normally would.

Obviously the implications of these "explanations" is that I think that a relationship that usually is mutualistic is being distorted here into a parasitic relationship (assuming that the plants do lose some energy resources by losing the nectar). My data is far from conclusive, but it does seem to suggest such a phenomena. Further studies are indicated, preferably earlier in the season. My own experimental design would be interesting to repeat in the spring--perhaps over a longer time period. Also, I would suggest some similar studies of suckers in a more natural environment. Data on the relationship between the ants and Populus grandidenta certainly seems worth pursuing further.

Before closing, I would like to clarify a final consideration --the reasoning behind including data on both the change in absolute amount of herbivory and the change in the percent herbivory. In dealing with the actual area gone to herbivores, I was intending to make a more concrete comparison based solely on the "amount eaten" over seven days, without regard to the overall leaf size, the original area herbivorized etc. This parameter can be reasonably used, only if one makes the assumption that the change in the area herbivorized is not a function of the inital area herbivorized. If the only change had been due to new herbivory, one could safely assume this. However, the leaves grew quite a bit over seven days and in many cases the area herbivorized seemed to grow porportionately without changing shape (see Figure 1). This suggests not further herbivory, but a "growth" of the herbivorized area as the plant grows. If one assumes that randomizing the

test and control plants will control for any differences this problem incurs, one can still use the change in absolute area as a parameter, but its strength is somewhat diminished because it does include this "growth" of herbovorized areas that is not due to new herbivory.

The change in percentage of herbivory parameter was designed to get around this problem. It is based on the assumption that the area herbivorized grows at the same rate as the leaf itself and as such does not constitute a larger percentage of the grown leaf than it did the smaller leaf. Thus, this parameter is probably more validly used here, but it does not have the benefit of implying how much actual biomass we are discussing.

Returning to the original hypotheses, it does not seem from the data noted here that the nectaries provide an alternative food source for the herbivores to decrease the loss of leaf area. However, the data does suggest that ants are attracted to the nectaries and that they may be decreasing the number of herbivores found on the plant. However, the nectaries do not seem to be causing a decrease in the amount or percent of herbivory over seven days in the plants that have them. Various properties of the ant/aspen reltionship may have bean altered in the site I studied, affecting the outcomeof the data. Based on those restrictions, I would not consider the hypothesis disproven that the nectaries cause a decreased rate of herbivory on the plants that bear them. Rather, I would suggest that the particular relationships I studied may represent unusual alterations of a more widely spread mutualistic relationship.

LITERATURE CITED

Janzen, Daviel H. Coevolution of mutualism between ants and acacias in Central America. Evolution, Vol. 20, No. 3, Sept. 1966.