DAMAGE INDUCED CHANGES IN THE FOLIAR
CHEMISTRY OF SUGAR MAPLE (ACER
SACCHARUM MARSH.) AND THEIR EFFECTS
ON THE FEEDING BEHAVIOR OF THE
FOREST TENT CATERPILLAR (MALACOSOMA
DISSTRIA HÜBNER)

by
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To my parents.

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INTRODUCTION

Rapid changes in foliage chemistry following damage
to nearby leaves has been observed in several species of
trees with the use of both chemical assays and bioassays
(Acer saccharum Marsh. and Populus xeuroamericana (Dode)
Guinier -Baldwin and Schultz 1983, Betula pubescens
Ehrh. var. tortuosa (Ledeb) Koehne -Haukioja and
Niemela 1979, Alnus rubra Bong. and Salix sitchensis
Sanson -Rhoades 1983). Because the chemical responses
of the leaves have included increases in such toxins as
phenolics and the protein-binding tannins, and the
bioassays have usually resulted in a negative impact on
the insect, these damage-induced changes have been
proposed as a defense against herbivory. Most studies
of induced defenses in trees have focused on the
detrimental effects they have on individual insects in
an attempt to show that these damage-stimulated changes
could alter the population sizes of the insects feeding
on inducible species. I feel however, that a response
which effects the feeding behavior of the insect could
have a much greater impact on the amount of foliage
consumed than a toxic reaction to tannins could. Toxic
or growth inhibiting effects of tannins can take some
time to develop during which insects will continue to
damage the tree. Caterpillars may even increase their

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consumption if forced to feed on previously damaged foliage in an apparent reaction to the drop in foliage quality the induced response produces (Fowler and MacGarvin 1986). Feeding deterrents in comparison, act immediately and directly in protecting foliage. Anyone who has ever seen a lone foliated tulip tree in a forest of gypsy moth denuded oaks cannot doubt the effectiveness of feeding deterrents. In addition, it has been proposed that in heterogeneously defended plants, such as would result in most situations involving damage-induced defenses, mortality could be increased by increasing the amount of time the insects spend searching for acceptable leaves (Shultz 1983). Movement has been shown to be attractive to predators and parasites (Richardson and Deloach 1972), and the chance of stumbling onto sedentary predators or pathogens is also increased with the area covered (Thornhill and Alcock 1983, Schultz 1983). Accidental falls from the foliage during breezes also can be a significant source of mortality even in the absence of movement by the insect (Raupp and Denno 1983). During my observations of caterpillars searching on branches in trees, it seemed that more insects fell off during winds when they were walking than when they were still.

In this study I address three questions. The first is whether previous damage to a sugar maple branch (Acer

saccharum Marsh.) has any effect on the feeding or searching behavior of a polyphagus insect, the forest tent caterpillar (Malacosoma disstria Hübner). The second question is whether a short-term damage-induced increase in the protein-binding tannin content of the leaves of sugar maples similar to the one observed in the lab (Baldwin and Schultz 1983) occurs in a natural setting. Finally, I determine if there is any correlation between the caterpillars behavior and any chemical response to damage by the tree.

To answer the behavioral response question, I observed the behavior of individual forest tent caterpillars on similar pairs of branches, one of which would have had some leaves damaged two days previous to the observation. I recorded the number of seconds the larvae spent feeding, moving about in search of food, and resting. In addition, I counted the number of times the larvae reared out from the branch. Rearing is done in an effort to catch hold of nearby foliage and move off of the branch the larvae is on. Along with searching bahavior, rearing indicates the level of dissatisfaction the larvae has for the foliage it is on (pers. obser.).

In addition to assaying the leaves for their protein-binding tannins, I also determined their free sugars content. Work on the taste perception of

Malacosoma americanum Fabr., a close relative of the forest tent caterpillar, has shown that it cannot directly perceive tannin, although tannin does decrease the caterpillars' ability to taste sugars (Dethier 1982). Since sugar concentration is an important quality in food selection for most caterpillars, this indirect effect of tannins could have a much greater behavioral significance than tannin content alone.

METHODS

Two types of data were generated in this study; (1) behavioral observations of forest tent caterpillars feeding on undamaged and previously damaged branches, and (2) analysis of the chemical condition of leaves from these branches. Behavioral data and leaf samples were collected in northern lower Michigan at The University of Michigan Biological Station at Pellston during May and June of 1986. The chemical analysis of the leaves was done at The University of Michigan, Ann Arbor. Five large sugar maples were sampled once a week during the study. Four small sugar maples were each used once as supplementary subjects. Eggs of the forest tent caterpillar were obtained from Dale Grisdale, Head of the Insect Rearing Lab of the Ontario Forest Pest Management Institute in Sault St. Marie, Ontario. The eggs had been collected during fall 1985, from a population in central Ontario.

Behavioral Data Collection

Behavioral data were collected on individual forest tent caterpillars feeding on pairs of branches, one with some leaves damaged and the other left undamaged as a control. Paired branches were taken from the same tree

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and from as similar microenvironmental conditions as was possible. All branches used contained five twigs, and treatment and control branches were chosen to be geometrically similar and essentially undamaged prior to the treatment. On the treatment branches, half of the leaves on the central twig (#3) were torn, one from each opposite pair of leaves. Two days later, both treatment and control branches were collected from the tree and immediately recut under water to re-establish their water columns. The branches were then brought into the lab and mounted in a horizontal position using a bar mount and test tube clamp. A second mount and clamp held a water filled test tube in which the cut end of the branch had been inserted while both were underwater, preventing the branch's water column from being broken.

Forest tent caterpillars were reared on an artificial diet (Bioserve, F9614) through the fifth molt at which time they were transferred to freshly collected sugar maple leaves. All larvae were allowed to feed on the leaf diet for at least three days before being used in a feeding trial. Throughout the experiment, the larvae were kept in a rearing chamber at 28°C and an eighteen-hour day.

On the morning of the behavioral observation, several larvae were selected randomly from the active individuals of a single cohort, the number depending on

the number of branch pairs to be tested that day, and placed in a jar with fresh sugar maple leaves until tested. The control and treatment branches from each pair were presented to the insects in a random order. A forest tent caterpillar which had been allowed to feed for five minutes on the foliage in the holding jar was placed on the center twig of the branch (twig #3), at the point where the petioles of the lowest leaf pair attached to the twig. This position was chosen so that the larva would not be biased in its decision by the presence of a leaf directly available to it, and also because this position allowed access to damaged leaves, undamaged leaves adjacent to damaged leaves, as well as leaves from other twigs.

As soon as the larva became active again, the observations began. The behavior and position of the larva was recorded continuously for thirty minutes. If the larva was immobile for more than ten minutes, this was noted and the trial was repeated later when it resumed activity. The forest tent caterpillar has regular cycles of activity throughout the day, alternating forty-five minutes to an hour of feeding followed by an equal period of rest. The first behavioral observation of the pair was run during the mid-to-late morning period of activity, while the second observation was done in the afternoon. After the

observations of the larva's behavior on both the damaged and the control branches were finished, the larva was returned to the jar containing the rest of its cohort. The larvae were kept in small groups from the same cohort because space limitations prevented keeping each larvae in a separate jar.

As soon as the observational trial on a branch was finished, the leaves were stripped off and placed into glassine envelopes. Each twig's leaves were bagged separately, as were the previously damaged and undamaged leaves from twig #3 on the treatment branch. The bagged leaves were frozen in liquid nitrogen to halt any biological processes. After freezing, the leaves were weighed, dried in a drying oven at approximately 70°C overnight, and then weighed again. The difference between the wet and dry weights represents the water content of the leaves for each twig. The leaves then were stored in a dry place away from the light until the chemical tests to determine the tannin and free sugar content of the samples were performed.

Chemical Data Collection

Prior to extraction, the leaves (excluding the veins and petioles) were ground to a powder using a Wiley mill with a #40 gauge screen. A sample consisted of the leaves from a single twig, except for twig #3

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from the experimental branches from which damaged and undamaged leaves were kept as separate samples.

The assay used to determine the amount of extractable tannin was the Hagerman radial diffusion method (Hagerman 1987). In this test, both hydrolyzable and condensed tannins were extracted using a 50% methanol solution. Eight ul of the extraction were then placed in a well in a plate of agar containing bovine serum albumin (BSA). In this assay, a cloudy ring is produced when the tannins precipitate the BSA, and the diameter of the ring is proportional to the total amount of protein-binding tannin in the sample. The free sugar content of the leaves was determined so that the sugar/tannin ratio could be calculated for each sample. This was done using the anthrone colorimetric method for determination of total carbohydrates (Tetley 1974) on a hot water extract of the powdered leaves. A description of the procedure for both the Hagerman radial diffusion method for determining tannin content and the anthrone colorimetric test for total carbohydrates is detailed below.

Determination of Tannin Content.

Reagents. All reagents were of analytical grade. Agarose, Type I; and bovine serum albumin (BSA), fatty acid free fraction V (A 6003); were obtained from Sigma

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Chemical Co. (St. Louis, MO). Buffer A was a 50 mM acetate buffer (pH 5.0) containing 60 uM ascorbic acid. For one liter of buffer A, 0.01057 g of ascorbic acid and 3.005 ml of 100% acetic acid were dissolved in 1 l of deionized water, and the pH was adjusted by the dropwise addition of sodium hydroxide.

Preparation of Gels. A 1% (w/v) solution of Agarose in buffer A was prepared by heating the mixture to boiling while stirring. The solution was then allowed to cool to 45°C with constant stirring. When the solution had reached 45°C, 0.1% (w/v) of BSA was mixed in and allowed to dissolve. For approximately 20 plates, 2 g of Agarose and 0.2 g of BSA were dissolved in 200 ml of buffer A. The solution was dispensed in 9.5-ml aliquots into plastic Petri dishes (8.5 cm diameter) and allowed to cool on a level surface. The plates then were stored in a refrigerator at 5.5°C. When the plates were used, five or six 4.5-cm diameter wells were punched in the agar, 1.5 cm. distant from each other and from the wall of the dish. The number of wells depended on whether the plate was to be used for the samples from a control branch or a damaged branch, since all twig samples from a single branch were tested on the same agar plate for logistical reasons.

Production of Tannin Calibration Curve. A stock solution containing 125 mg of tannic acid/ml was

prepared in 50% methanol. This solution then was diluted so that the concentration of tannic acid in the calibration samples ranged from 0.5 mg/8 ul to 0.02 mg/8 ul, with intervals of 0.1 mg/8 ul above 0.2 mg/8 ul, and 0.02 mg per aliquot below this concentration. Eight ul aliquots of the calibration solutions were applied to individual wells in an Agarose/BSA plate, the plates were sealed with Parafilm and placed in an incubator set at 30 C. One hundred and twenty hours later, two diameters of the resultant rings were measured at right angles to each other. The two diameters were averaged and this value then was squared and used to construct a curve of squared average ring diameter vs. the concentration of tannic acid present in the calibration sample. This curve was later used to determine the concentration of tannin (expressed as tannic acid equivalents, TAE) in leaf extract samples.

Extraction of Leaf Tissue. One hundred mg of leaf powder from each sample was extracted for one hour with 0.5 ml 50% (v/v) methanol. The samples were then centrifuged at 5000xg for fifteen minutes in order to remove the solid material from the extract which interfered with the pipeting of the sample.

Assay Method. An aliquot of 8 ul of the supernatent solution from the centrifugation was added to the appropriate well in the agar plate. After all

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the samples had been placed in the wells, the plates were covered and incubated at 30°C for one hundred and twenty hours. Two diameters, taken at right angles to each other, were measured for each ring. The average diameter was calculated and this value was squared for comparison with the calibration curve of known tannin concentrations to determine the concentration of tannin in TAE for that extract sample. The resultant value was converted to its equivalent percent of the sample's dry weight.

Determination of Free Sugar Content.

Reagents. The concentrated sulfuric acid (680-2), thiourea (T 7875), and anthrone (A 1631) were all of analytical quality and were obtained from SIGMA Chemical Suppliers (St. Louis MO) as was the glucose (G 5000) used for producing the calibration curve. Filter paper used was #42 Whatman, (4.25 cm diameter).

Preparation of the Anthrone Reagent. The anthrone reagent consisted of a solution of 0.05% anthrone, 1% thiourea, and 66% sulfuric acid in deionized water. To make the reagent, 280 ml of chilled, deionized water and 666 ml of sulfuric acid were mixed in a flask placed in an ice water bath. The acid was added slowly while the solution was swirled to prevent excessive heat and fumes from being produced during the mixing. After the

solution had cooled to room temperature, 10 g of thiourea was added followed by 0.5 g of anthrone. The reagent was stored in a refrigerator at 5.5°C. Fresh reagent was prepared every two weeks.

Water Extraction of the Leaves. A sample of ground leaf tissue weighing 0.025 g was mixed with 15 ml of deionized water in a large test tube. The mixture was placed in a boiling water bath for two hours. Deionized water was added every twenty minutes to replace the liquid lost to evaporation. After the extract had cooled, it was filtered at reduced pressure through #42 Whatman filter paper. The residue was washed three times with deionized water, and this liquid was added to the rest of the filtrate. The filtrate was then made up to 25 ml in a volumetric flask by the addition of deionized water.

Production of the Calibration Curve. A separate calibration curve was produced for each group of samples which were put through the anthrone test together. This was necessary because the color development during the incubation of the anthrone reagent with the leaf extract was very rapid. Even a small difference in the length of the incubation could lead to different absorbance values for the same sugar concentration. For this reason, the absorbance value for a sample could only be converted to a sugar concentration using a calibration

curve produced during the same assay. The calibration curve was produced using dilutions of a stock aqueous solution of 0.25 mg of glucose per ml. This stock solution was diluted to produce a range of glucose solutions having concentrations from 5 mg/2ml solution, to 25 mg/2 ml solution, increasing by increments of 5 mg. These calibration solutions were subjected to the anthrone proceedure along with the group of extract samples being assayed. The absorbance values produced were regressed to make the calibration curve for that batch of samples. Along with the glucose solutions and extract samples, a pure water sample was tested with the anthrone assay, as a blank with which to calibrate the spectrophotometer prior to doing the readings of the other solutions.

Anthrone Assay for Free Sugars. Leaf extract samples of 0.5 ml were combined with 5 ml of anthrone reagent in separate test tubes. The solutions were then vortexed and cooled in an ice bath. After cooling, the samples were vortexed again and placed in a boiling water bath for exactly eight minutes. All samples were vortexed once during the heating period. The reaction mixtures were then rapidly cooled to room temperature in an ice bath, and mixed again on the vortex mixer prior to reading their absorbance value at 600 nm on a Zeiss PMQII spectrophotometer. The values produced for the

extract samples were compared with the calibration curve to determine their free sugar content and this value was converted to its equivalent percentage of the dry weight of the leaf sample.

RESULTS

Behavior of Larvae

Forest tent caterpillars were observed to spend significantly more time feeding on the damaged branches than on the controls (Table 1). Searching behavior and escape behavior such as rearing, were significantly more common on the control branches (Table 1). The amount of time the larvae spent resting however, was nearly equivalent on both branch treatments (Table 1).

The time of day the observations took place did not significantly affect the amount of feeding on either the damaged or control branches (Table 2). The time of day did not affect the amount of searching in the damaged branches. However, there was a near significant difference (P <0.07) in the amount of time searching on the control branches depending on when the trial was run (Table 2). Behavioral observations on control branches run in the morning showed a higher mean search time than those run in the afternoon.

Within the branches, the time spent feeding on the third twig was greater than for any other twig in the damaged and control branches (Tables 3 and 4). This difference was significant in the damaged branches, but in the control branches, the times spent feeding on

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Table 1 - Comparison of time spent by forest tent
caterpillars in feeding, searching, and resting, and the
amount of rearing on damaged and control branches of sugar
maple. Values denoted by the same letter do not differ at
the P < 0.05 level according to a paired T test.

Behavior	N	Damaged	Control	P
Feeding (sec/30min)	40	928.8 a (+/- 584.8)*	674.5 b (+/- 571.8)	0.029
Searching (sec/30min)	40	681.8 c (+/- 518.5)	927.5 d (+/- 531.9)	0.023
Rearing (rears/30min)	40	4.4 e (+/- 6.0)	9.2 f (+/- 11.2)	0.017
Resting (sec/30min)	40	305.3 g (+/- 373.0)	303.8 g (+/- 415.7)	0.99

* Standard deviations are presented in parenthesis below
   the mean.

Table 2 - Comparison of behavior of forest tent caterpillars on sugar maple within branch treatments in morning and afternoon observations. Values denoted with the same letter do not differ significantly at the $P < 0.05$ level according to a Student t test.

Behavior	Treatment	Morning	Afternoon	P
Feeding (sec/30min)	Damaged	1020.6 a (+/- 569.3)*	845.0 a (+/-619.4)	0.37
	Control	591.5 b (+/- 550.3)	430.6 b (+/- 514.7)	0.43
Searching (sec/30min)	Damaged	595.6 c (+/- 426.8)	647.9 c (+/-517.3)	0.74
	Control	1092.0 d (+/- 526.5)	778.5 d (+/- 438.0)	0.09

* Standard deviation is presented in parenthesis below the mean.

Table 3: Comparison of time spent feeding by forest tent caterpillars on twigs of damaged branches of sugar maple. Two stars (**) indicate that the mean number of seconds feeding on the two twigs were highly significantly different (P < 0.01) according to an analysis of varience.

Twig Position (low near trunk)	Feeding (sec.)	Probability of Significance (P) for the Comparison of the Two Means — Twig Number				
		1	2	3	4	5
1	88.1 (+/- 310.8)*	NA	0.52ns	0.00**	0.85ns	0.80ns
2	144.1 (+/- 452.8)		NA	0.00**	0.64ns	0.65ns
3	491.3 (+/- 600.6)			NA	0.00**	0.00**
4	101.6 (+/-344.8)				NA	0.98ns
5	105.1 (+/-285.1)					NA

* Standard deviations are presented in parenthesis below the mean.

Table 4: Comparison of time spent feeding by forest tent caterpillars on twigs of control branches of sugar maple. Values denoted with a * or ** indicate that the mean number of seconds feeding on the two twigs were significantly or highly significantly different, respectively, at $P < .05$ according to an analysis of varience.

Twig Position (low near trunk)	Feeding (sec.)	Probability of Significance (P) for a Comparison of the Two Means				
		Twig Number				
		1	2	3	4	5
1	37.3 (+/- 155.5)*	NA	0.23ns	0.00**	0.18ns	0.20ns
2	114.0 (+/- 370.0)		NA	0.06ns	0.97ns	0.99ns
3	298.5 (+/- 499.8)			NA	0.05*	0.06ns
4	111.5 (+/- 308.5)				NA	0.98ns
5	113.3 (+/- 339.6)					NA

* Standard deviations are presented in parenthesis below the mean.

twigs 2 and 5 were high enough so that they just exceeded the 5% limit for significance. Twigs trunkward and tipward of the center twig were not significantly different in the amount of time spent feeding on them (Tables 3 and 4). Feeding on the damaged and undamaged leaves of the damaged twig 3 in the treatment branches was not significantly different (Table 5). Average feeding periods for the equivalent twigs on control and treatment branches (treatment twig #1 with control twig#1, etc.) were not significantly different (Table 6).

Searching within the damaged branches was significantly concentrated on the damaged twig (Table 7). However, within the damaged twig there was no difference in the amount of time spent on the damaged and undamaged leaves. In the control branches, the mean values for the time spent searching on the twigs were not as distinctly grouped as they were in the damaged branches. The time spent searching on twig 3 was greater than for any other twig, but it was not separable from the values for either twigs 2 or 5 (Table 8). Searching time on twigs in the same positions on damaged and control branches (i.e. treatment twigs #1 with control twigs #1, etc.) were statistically inseparable for twigs 1,2,3, and 4 (Table 9). On control branches, much more time was spent searching on

Table 5 - Comparison of time spent by forest tent
caterpillars feeding and searching on the damaged and
undamaged leaves of the third twig of the treatment
branches of sugar maple. Values denoted by the same letter
do not differ significantly at the $P < 0.05$ level according
to an analysis of varience.

Behavior	N	Damaged	Undamaged	P
Feeding	40	224.0 a (+/- 447.2)*	267.3 a (+/- 529.2)	0.69
Searching	40	166.9 b (+/- 250.2)	138.1 b (+/- 155.5)	0.54

* Standard deviations are presented in parenthesis below
  the mean.

Table 6 - Comparison of time spent feeding by forest tent caterpillars on twigs of the same position on damaged and control branches of sugar maple. Values denoted by the same letter do not differ significantly at the $P < 0.05$ level according to a Student T test.

Twig Position	Feeding on Damaged Branches (sec.)	Feeding on Control Branches (sec.)	P
1	88.1 a (+/- 311.4)*	37.3 a (+/- 154.9)	0.36
2	144.1 b (+/- 458.3)	114.0 b (+/- 374.5)	0.75
3	491.3 c (+/- 600.0)	298.5 c (+/- 500.0)	0.12
4	101.6 d (+/- 346.4)	111.5 d (+/- 308.2)	0.89
5	105.1 e (+/- 284.6)	113.3 e (+/- 346.4)	0.91

* Standard deviations are presented in parenthesis below the mean.

Table 7 - Comparison of time spent searching by forest tent caterpillars on the twigs of the damaged branches of sugar maple. Twig searching times were considered to be statistically different at P <.05 as determined by an analysis of varience. Two stars (**) indicate the mean number of seconds searching on the two twigs are highly significantly different (P < 0.01).

| Twig Position (low near trunk) | Searching (sec.) | Probability of Significance (P) for the Comparison of the Two Means | | | | |
| | | Twig   Number | | | | |
		1	2	3	4	5
1	66.13 (+/- 149.7)*	NA	0.83ns	0.00**	0.64ns	0.55ns
2	75.1 (+/- 213.9)		NA	0.00**	0.88ns	0.75ns
3	305.0 (+/- 315.5)			NA	0.00**	0.00**
4	81.4 (+/- 141.1)				NA	0.83ns
5	90.0 (+/- 201.6)					NA

* Standard deviations are presented in parenthesis below the mean.

Table 8: Comparison of time spent searching by forest tent caterpillars on the twigs of control branches of sugar maple. Values denoted by a * or ** indicate that the mean number of seconds searching on the two twigs are significantly or highly significantly different, respectively, at $P < 0.05$ as determined by an analysis of varience.

| Twig Position (low near trunk) | Searching (sec.) | Probability of Significance (P) for the Comparison of the Two Means | | | | |
| | | Twig Number | | | | |
		1	2	3	4	5
1	79.8 (+/- 169.7)*	NA	0.15ns	0.00**	0.23ns	0.04*
2	158.3 (+/- 294.0)		NA	0.09ns	0.69ns	0.58ns
3	268.5 (+/- 274.6)			NA	0.02*	0.25ns
4	134.8 (+/- 228.9)				NA	0.31ns
5	195.3 (+/-299.8)					NA

* Standard deviations are presented in parenthesis below the mean.

Table 9 - Comparison of time spent searching by forest tent caterpillars on twigs of the same position on damaged and control branches of sugar maple. Values denoted with the same letter do not differ significantly at the $P < 0.05$ level as determined by an analysis of variance.

Twig Position	Searching on Damaged Branches (sec.)	Searching on Control Branches (sec.)	P
1	66.1 a (+/- 148.3)*	79.8 a (+/- 170.3)	0.70
2	75.1 b (+/- 214.5)	158.3 b (+/- 293.3)	0.15
3	305.0 c (+/- 316.2)	268.5 c (+/- 273.9)	0.58
4	81.4 d (+/- 141.4)	134.8 d (+/- 228.0)	0.21
5	90.00 e (+/- 202.5)	195.3 e (+/- 300.0)	0.07

* Standard deviations are presented in parenthesis below the mean.

the fifth twigs than was spent on the same twigs on treatment branches. While this difference was not significant at P <0.05, it was close to significance with a P = 0.07.

Chemical Analysis of Leaves

Overall levels of tannins, free sugars, water and the sugar/tannin ratio in the damaged and control branches were not significantly different at P <.05 (Table 10). The sugar/tannin ratio was greater in the damaged branches and close to significance with a P < 0.10. Both control and damaged branches showed a decrease in the sugar/tannin ratio in the twigs towards the tip of the branch, but the slope of the regression line of sugar/tannin ratios vs. the distance from the base of the branch was steeper for the treatment branches than it was for the control branches (Fig.1).

Examination of the tannin levels separately show that tannin concentrations increase in twigs further from the trunk (Fig. 2). Mean tannin levels in twigs from the damaged branch were consistently lower than their control counterparts, but this difference was not significant.

Sugar concentrations of the damaged and control branches were highly variable and show no consistent trend with distance from the trunk (Fig. 3). The two

Table 10 - Comparison of levels of tannin(TAE), free sugar, sugar/tannin, and water for damaged and control branches of sugar maple. Values denoted by the same letter do not differ significantly at the P < 0.05 level according to a Wilcoxin ranked pairs test.

Character	N	Damaged	Control	P
Tannin(TAE) (% dry wt.)	38	4.9 a (+/- 1.9)*	5.0 a (+/- 1.9)	0.42
Free Sugar (% dry wt.)	38	7.4 b (+/- 1.3)	7.4 b (+/- 1.6)	1.00
% Sugar/ % Tannin	38	2.0 c (+/- 1.0)	1.7 c (+/- 0.6)	0.10
Water (% fresh wt.)	38	54.7 d (+/- 7.8)	55.5 d (+/- 8.5)	0.42

* Standard deviations are presented in parenthesis below the mean.

Figure 1. Mean % free sugar/% tannin (TAE) of dry weight
           for twigs from damaged and control branches.
           Values are plotted against the mean distance of
           the twigs from the base of the branch.

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Figure 2. Mean % tannin (TAE) of dry weight for twigs from
            damaged and undamaged branches. Values are
            plotted against the mean distance for those twigs
            from the base of the branch.
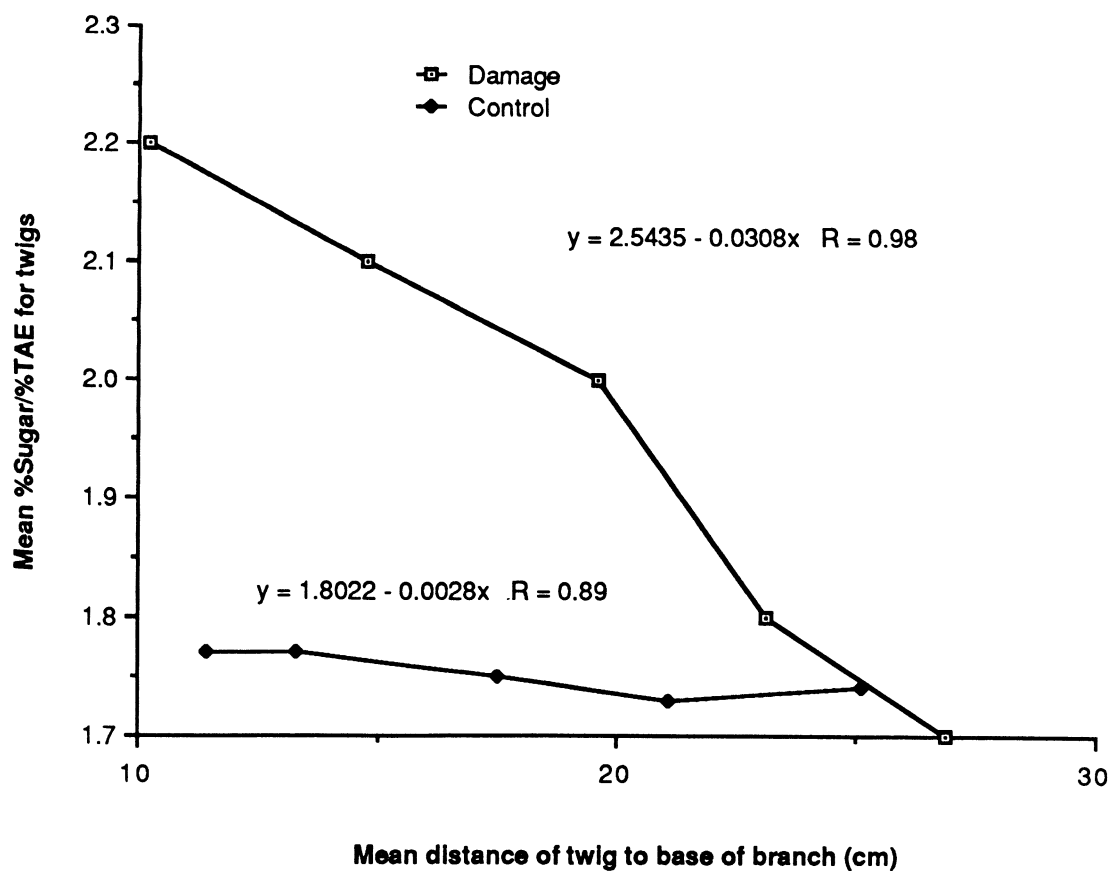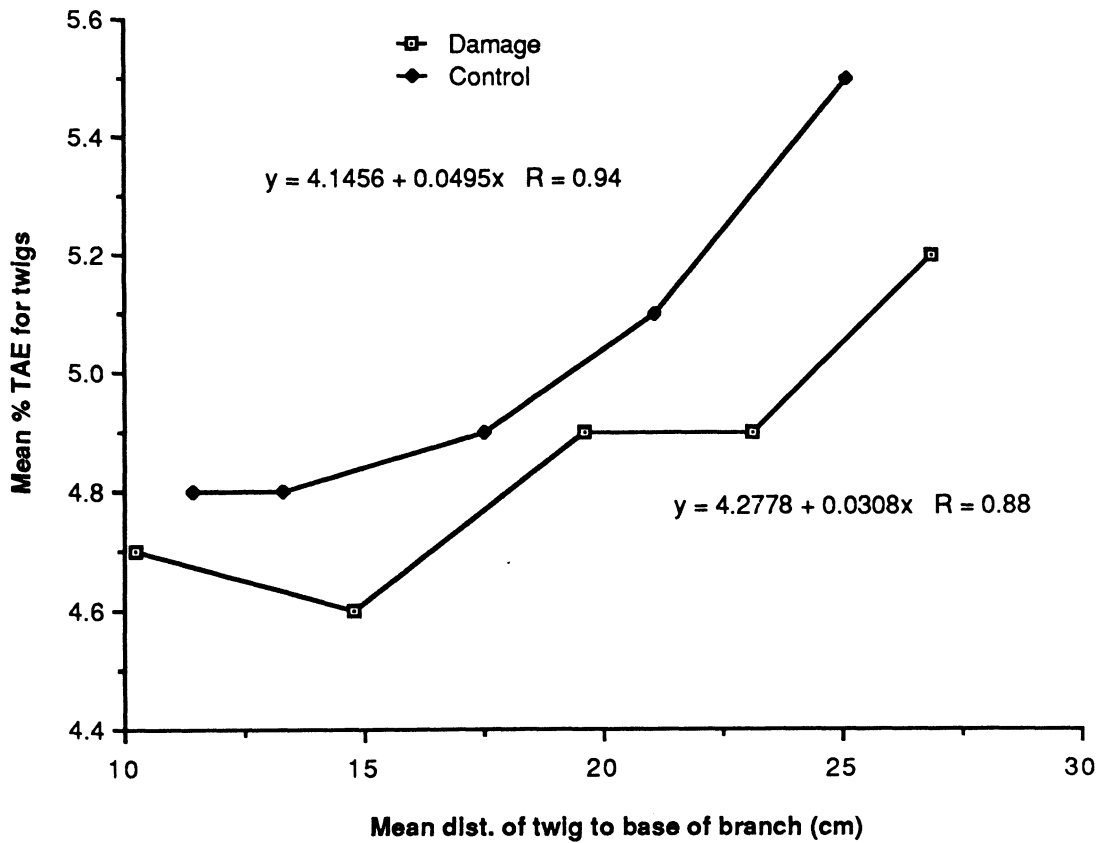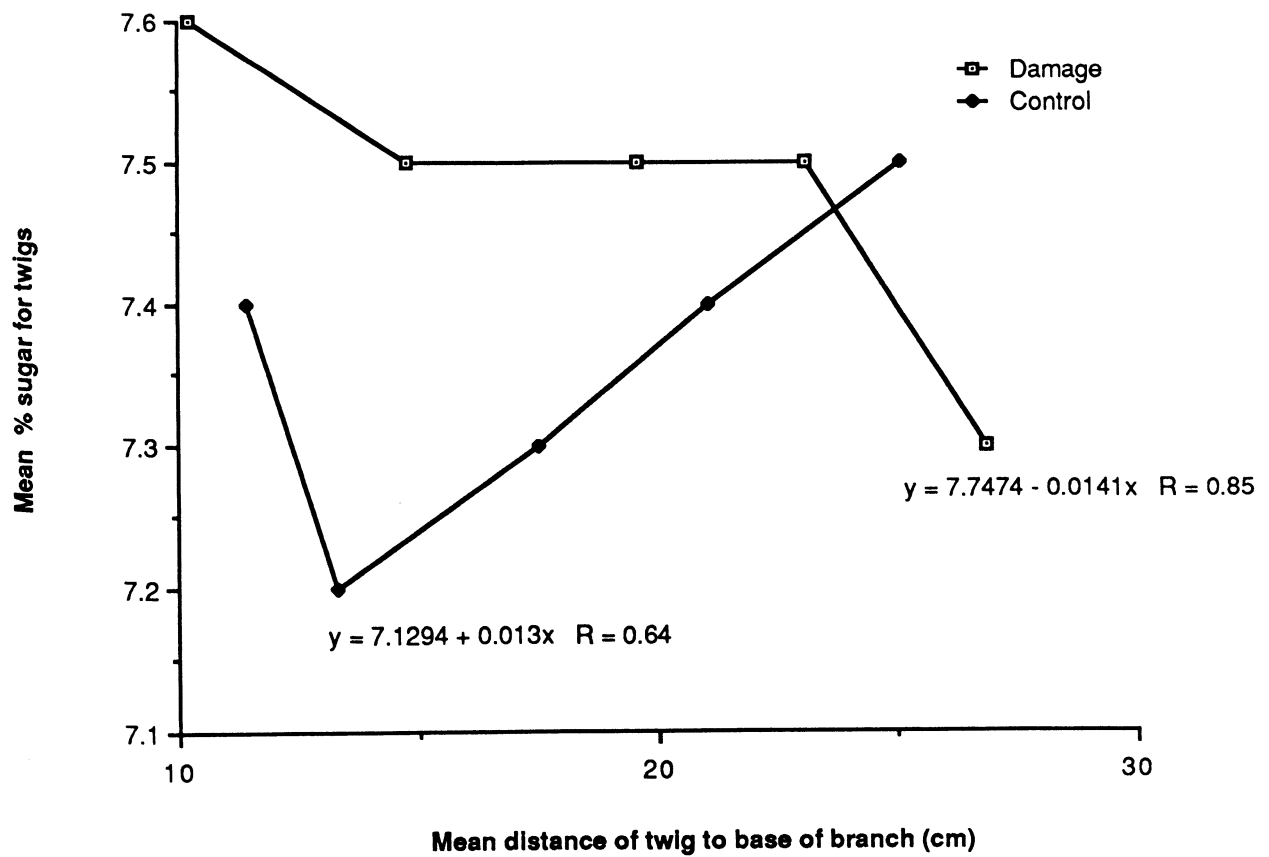
Figure 3. Mean % free sugar of dry weight for twigs from damaged and undamaged branches. Values are plotted against the mean distance for those twigs from the base of the branch.

curves are not significantly different.

While the difference between the water contents of leaves from damaged and control branches was not significant (Table 10), there was some effect on the water concentration in twigs within the damaged branches. Twigs in position 2, just trunkward of the damaged twig, and which shared all of their vascular system with that twig, had much lower water levels than those twigs at position 2 which did not share vascular systems with the injured leaves (Fig. 4). Twigs at the other positions on the damaged branch did not show any response in their water content to the injury, regardless of whether they shared vascular tissue with the damaged twig.

Neither feeding nor searching times were significantly correlated with either tannin, free sugar, water or sugar/tannin ratio. This result was true for both treatment and control branches (Table 11).
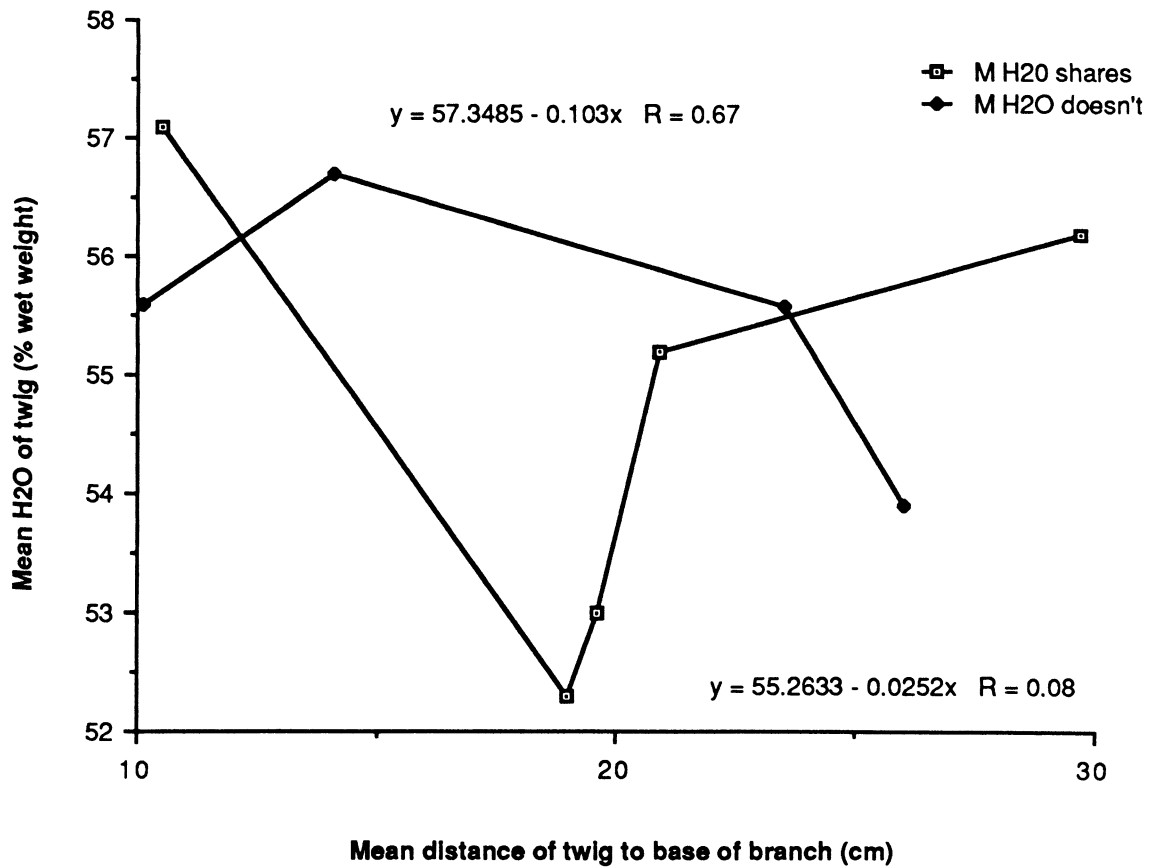
Figure 4. Mean % water content for twigs sharing and not
        sharing their vascular systems with the damaged
        twig. Values are plotted against the mean
        distance of those twigs from the base of the
        branch.

Table 11 - Correlation between the levels of tannins (TAE), free sugars, sugar/tannin, and water in sugar maple leaves and the feeding and searching behavior of forest tent caterpillars. The designation NS indicates there was no correlation between the behavior and the leaf characteristic according to a simple linear regression.

	Probability of Significance (P) for Correlation of Character with Behavior		
		Behavior	
Leaf Character	N	Feeding	Searching
Tannin(TAE) % dry wt.	38	0.42 NS	0.46 NS
Free Sugar % dry wt.	38	0.16 NS	0.26 NS
Water % fresh wt.	38	0.30 NS	0.26 NS
Sugar, Tannin Ratio	38	0.68 NS	0.56 NS

DISCUSSION

Behavioral Responses of Larvae to Leaf Damage

Damage to even a few leaves on a sugar maple branch
seems to radically increase its acceptability as food
for the forest tent caterpillar as is indicated by the
increased consumption on the damaged branches. Other
evidence of the increased palatibility of the damaged
branches is the decreased searching time on the treated
branches. In contrast, when the larvae were on the
control branches they not only spent more time
searching, but their search area was wider as indicated
by the lack of significant difference between the amount
of time spent searching on twigs 2,3, and 5. The large
amount of time spent on twig #5 also reflects attempts
by the larvae to leave the branch altogether, as does
the significantly greater amount of rearing observed on
the control branches. Both hanging from the tip of the
branch and rearing up to catch overhanging leaves were
common methods used by forest tent caterpillars to move
onto different branches in the field. The decrease in
these behaviors on the damaged branch indicates an
increase in the palatability of the damaged branch.

The increased feeding by the forest tent
caterpillars on damaged branches is most intriging,

since several researchers have postulated that the changes in leaf characteristics as a result of damage are antiherbivore defenses (Baldwin and Schultz 1983, Haukioja and Niemela 1979, Wratten et. al. 1984). In this interaction, the injury induced changes decreased the defended condition of the foliage to the forest tent caterpillar, a result which would lead to an even greater level of damage to the tree than if it possessed no response to damage at all.

I propose that there could be three possible explanations for the observed behavior of the forest tent caterpillars to previously damaged sugar maple foliage: 1) There were some chemical characteristics of the leaves that had been altered as an effect of the damage and which increased the palatibility of the induced foliage. 2) It was relatively easier for the larvae to initiate feeding on the tears in the damaged leaves. 3) There was a consistent difference in the level of hunger of larvae during observations on the damaged and control branches.

Chemical Response of Tree to Damage

While the sugar/tannin values for the damaged and control branches were nearly significantly different, they were not correlated with either feeding or searching behavior. In addition, the differences in the

sugar/tannin ratios were a random effect and not a deliberate response by the tree to damage. Neither the sugar nor the tannin concentrations were affected by damage, and this would be a prerequisite for the tree to have any control over the sugar/tannin ratio.

The protein binding tannin concentration for the damaged branches was not significantly different from that of the control branches, indicating there was no induced effect of damage in this character. A comparison of the tannin levels in twigs within the damaged branches was done, and it showed no indication of any local effects on the tannin concentration near the site of damage. This was surprising since Baldwin and Schultz (1983) had found significant increases in tannin content 48 hours after damage in sugar maple. Two explanations for this discrepancy are possible. The first is that a compound which triggers the changes in the tannin level is being released into the air by damaged cells, but the amounts are so small that they normally affect only the tissue immediately adjacent to the damage. Such a limited response would be unlikely to be detected in a sample of several leaves, such as in my study. In the enclosed growth chambers that Baldwin and Schultz (1983) used, this triggering compound may have built up to levels where it could cause increases in tannins in distant leaves. This response would be

impossible in my field study because constant breezes
would quickly disperse any chemical released into the
air. An alternative explanation is that the results
observed in the laboratory study were due to some
outside factor and not from the damaged plants and that
there was no damage-induced increase in tannin
concentration.

The lack of consistent trends and the similar
degrees of variability of the free sugar levels in both
the damaged and control branches indicates that there is
no defensive alteration of this characteristic as a
result of damage. I expect that these results are due
to differences in the microclimate the branches were
exposed to before collection. A study of the sugar
content in sugar maple leaves has shown that this
characteristic is typically highly variable, and greatly
influenced by temperature and light intensity, even
under short durations (Donnelly 1976).

The water content of certain twigs within the
damaged branch were affected by that treatment.
However, this response seems to be a passive result
rather than an active, defensive reaction by the branch.
Only those twigs just trunkward and sharing all of their
vascular system with the damaged twig showed a decrease
in water content (Fig. 4). This is a result that could
be expected since the injured leaves would have a very

high rate of evaporation from torn tissue. The draw of water into the damaged leaves would be increased and this could locally deplete the fluid in the xylem bringing water from the roots. Twigs nearer to the damaged twig would be expected to share a greater percentage of their vascular system with that twig, and those undamaged twigs which originated from the branch at the same angle as the injured twig should be expected to share even more. When water was depleted from the xylem used by the damaged twig, I would expect to see a decrease in the water content of twigs proportional to the degree to which they were dependent on the depleated xylem. This is exactly what I observed.

Ease of Feeding

Another factor which could have influenced the increase in feeding was the apparent ease of initiating feeding on a tear compared with the difficulty the larvae seemed to have in biting through a natural leaf edge. If ease of feeding was an important factor in the increase in consumption on the injured branches, I would expect that the amount of time spent eating on the damaged leaves would be greater than that on the undamaged leaves from the same twig. However, when this was tested, I found no difference in the amount of time feeding on either the damaged or undamaged leaves of the

treatment branches third twigs. For this reason I must
conclude that the relative ease of feeding at the damage
sites was not a significant factor influencing the
behavior of forest tent caterpillars.

Differences in Hunger

The third possible explanation for increased
feeding on damaged branches is that there was a
difference in the degree of hunger, and perhaps also in
the selectiveness, of the larvae during the observations
on the injured and control branches. The larvae were
never without food, but fresh leaves were supplied only
once a day in the morning. If the larvae were not
eating the leaves provided after a certain time, they
could have been more hungry in the mornings than at
other times of the day. More of the damaged branch
trials than the controls were done in the morning and if
the animals were more hungry during that time this could
lead to the greater feeding observed in the damaged
branches. Selectivity could also have been decreased by
hunger, which could explain why there was less searching
on the damaged branches than on the control. There has
been one study of lepidopteran behavior which showed a
decrease in the degree of selectivity with an increase
in the time from the last satisfaction (Singer 1982),
and while the study only dealt with adults, it seems to

indicate the possiblity that such behavior could exist in the larvae.

I tested this hunger theory by comparing the time spent feeding and searching within branch treatments between observations done in the morning and the afternoon. I found that levels of feeding did not vary significantly with the time of day the observation was done. In addition, searching on the control branches was higher in the morning, and nearly significantly so, which would argue against the idea that the larvae are less selective at this time. These results indicate that hunger is not important in determining the differences in consumption on the damaged and control branches.

Corrolations Between Behavior and Leaf Chemistry

The lack of correlation between the forest tent caterpillars behavior and the leaf characteristics of tannin and free sugar was not surprising given that behavior showed a significant response to damage while those characters did not. However, the lack of correlation between free sugar/tannin ratio and feeding was unexpected. In the eastern tent caterpillar, a close relative of the forest tent caterpillar, tannin has been found to inhibit the larvae ability to perceive sugar (Dethier 1982). Since sugar is a feeding

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stimulant, I had expected that as the sugar/tannin ratio decreased, the feeding by the larvae should also decrease and vice versa. Apparently, other factors are more important in determining where forest tent caterpillars will feed than the perceived sugar concentration.

CONCLUSIONS

While the behavioral bioassay indicated that there were rapid changes in the leaves of sugar maple in response to damage, the chemical results of my field study were not similar to the ones observed in the laboratory setting (Baldwin and Schultz 1983). Chemical changes observed in this study did not include any changes in the levels of protein-binding tannins as had been previously observed in the laboratory. The behavioral response by the forest tent caterpillars to the induced foliage was also surprising given the results of work done on perception and food choice in a closely releated species. In summary, injury to leaves of the sugar maple induces certain unidentified changes to occur in the foliage which stimulate feeding in forest tent caterpillars. This result indicates that the damage-induced changes in sugar maple do not function as a feeding deterrent in the interaction of this tree and the larvae of the forest tent caterpillar.

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