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Limiting Effects of Low Leaf-Water Content on the Nitrogen Utilization, Energy Budget, and Larval Growth of *Hyalophora cecropia* (Lepidoptera: Saturniidae)

J. Mark Scriber*
Department of Entomology, Cornell University, Ithaca, NY 14853, USA

Summary. *Hyalophora cecropia* larvae were reared on leaves of wild cherry, *Prunus serotina,* which contained variable amounts of leaf water but otherwise did not differ in fiber, total nitrogen, and caloric content. Larvae which were fed leaves low in leaf water grew more slowly and were less efficient at utilizing plant biomass, energy, and nitrogen than those larvae fed leaves which were fully supplemented with water.

Experiments were performed using excised leaves under different regimes of relative humidity and leaf water supplementation in climatic control chambers maintained at identical temperatures and photoperiod. Foodplant biomass utilization efficiencies were severely reduced by decreasing amounts of leaf water. Growth rates were halved and the efficiency of conversion of assimilated dry matter into larval biomass was reduced from 82% in the treatment with fully supplemented leaves to 34% in the driest treatment. The nitrogen utilization efficiency (N.U.E.) was reduced from 75–80% to 48%, and the relative accumulation rate of nitrogen (N.A.R.) was suppressed nearly 2-fold for larvae on low-water leaves. Relative maintenance costs (calories expended in respiration/mg tissue/day) of larvae were nearly five times higher on dry leaves than on fully supplemented leaves. Larvae on leaves which were low in water content were themselves more desiccated, and metabolized greater portions of assimilated energy, perhaps in an attempt to supplement body water with metabolic water derived from respiration.

The larval rates of consumption of biomass, energy, and nitrogen were the same for all treatments, indicating that leaf water affected larval growth primarily by restricting the efficiency of utilizing these nutrients. Where water was limiting (as in tree leaves), an increased consumption rate did not appear to be a successful means of increasing growth rates. There were daily and seasonal differences in leaf water content between different trees of the same species. Although absolute differences in leaf water exist between

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* *Present address:* Department of Biological Sciences, Wright State University, Dayton, OH 45431, USA

different trees and between young and old (fully expanded) leaves of a single tree, these differences are proportional and parallel each other through daily and seasonal cycles.

In spite of evolutionary adaptations of herbivores to acquire adequate water and avoid desiccation, the leaf water content naturally encountered by cecropia larvae on cherry leaves may limit their growth, especially if the R.H. is low.

Introduction

Among the barriers facing leaf-feeding insects in their evolution from detritus-feeders is the problem of acquiring adequate nutritional water and avoiding desiccation (Southwood, 1972). Lepidoptera larvae generally maintain a body water content of 85–92% (c.f. Rudolfs, 1926; Evans, 1939; Wigglesworth, 1965; Bursell, 1970; Scriber, 1975), although the water content of leaves, especially mature tree leaves, is often substantially lower (McHargue and Roy, 1932; Feeny, 1970; Likens and Borman, 1970). Thus the water content of leaves may, under some circumstances, limit the growth of larvae despite their adaptations for feeding on leaves low in water.

Limiting effects of low plant water content on insect growth rates have been suggested previously (Waldbauer, 1962, 1964). Armyworm larvae, *Spodoptera (=Prodenia) eridania* Cram., have more difficulty eating and processing tree leaves than herb leaves (Soo Hoo and Fraenkel, 1966), although it was uncertain whether this was due to the high fiber content or the low water content of tree leaves. The net growth efficiency (or E.C.D. = efficiency of conversion of digested food) of the larvae of several Lepidoptera species was found to be correlated strongly with the leaf water content of their various food-plants (see Feeny, 1975); uncertainty again remains, however, as to whether low feeding efficiencies on tree leaves were caused by low water content or by other leaf characteristics, such as high fiber content or low nutritional value, which are themselves correlated with low water content.

Low relative humidity also decreases growth rate and pupal weight in certain Lepidoptera and Coleoptera (Ahmad, 1935; Holdaway, 1932; Sharada and Bhat, 1957a; Speicher, 1931). More food and energy were required for larval growth in low humidity regimes (Fraenkel and Blewett, 1944; Sharada and Bhat, 1957b). The effect of relative humidity on growth and survival of larvae with and without free drinking water was greatest at low humidities (Mellanby and French, 1958). The kinetics of water exchange (Arlian and Wharton, 1974) for an arthropod usually favor a net loss to the environment, and depend on the water vapour activity and the organism's critical equilibrium activity (Wharton and Devine, 1968). Murray (1968) found that larvae of the beetle *Tenebrio molitor* were able to survive a very dry period by not eating; larvae which ate lost more water in respiration than they gained in feeding and eventually died.

I undertook the present study to determine if leaf-water content is ever likely to limit the growth of a tree-feeding insect species under conditions of leaf water content and relative humidity which are encountered naturally in the field. I chose to study larvae of the cecropia moth, *Hyalophora cecropia* L., feeding on leaves of wild black cherry, *Prunus serotina* Ehrh. The larvae of this widely distributed saturniid are polyphagous, attacking a wide range of deciduous trees (Waldbauer and Sternburg, 1967; Forbes, 1969; Scarbrough, 1970; Ferguson, 1972; Scarbrough et al., 1974). Near Ithaca, New York the major food-plants are *P. serotina, Malus* spp. (apple), *Ulmus americana* (elm), *Acer saccharum* (sugar maple), *Sambucus canadensis* (elderberry) (Forbes, 1969; J.G. Franclemont, personal comm.). Larvae of the single annual generation (Forbes, 1969; Waldbauer and Sternburg, 1973) feed from late June through September (Sternburg and Waldbauer, 1969).

Methods and Materials

Feeding Experiments with Larvae

I reared cecropia larvae[1] from eggs laid by a wild female captured on the Cornell University campus in Ithaca, New York. Larvae were fed freshly collected *P. serotina* leaves daily throughout the first three instars. Freshly molted fourth (penultimate) instar larvae were selected for the feeding experiments.

The feeding experiment included four treatments. Two biochambers were maintained at approximately 21.6°C (23°:19°C day:night) and a 16:8 hour photoperiod. The mean relative humidity of one chamber was 75% and of the other, 35%. Twelve weighed larvae in individual plastic petri dishes (150 × 25 mm), with weighed *P. serotina* leaves were placed in each chamber. Within each of the two biochambers, the water supply of the leaves in 6 of the dishes was supplemented by passing the petiole through a small hole in the wall of the dish and into a florist's "aqua pic"® (Syndicate Sales, Kokomo, Indiana). The "aqua pic" is a plastic vial full of water and sealed by a rubber cap. It does not leak because the petiole passes through a small hole in the rubber cap. The four treatments were: 75% R.H. with and without supplementation (75+ and 75− respectively) and 35% R.H. with and without supplementation (35+ and 35− respectively).

Freshly molted 4th instar larvae were weighed and given a known weight of fresh food. Approximately every 30h thereafter fresh leaves of known weights were added, fresh weights of the larvae recorded, and the feces removed. Food consumption was estimated by a standard gravimetric technique (Waldbauer, 1968). The dry weight gained by experimental larvae was estimated by multiplying their gain in fresh weight by the mean % dry matter of an additional six larvae subjected to each treatment and sacrificed at intervals.

Plants, larvae, and feces were frozen and freeze-dried for dry weight determination and in preparation for caloric and nitrogen determinations. Caloric content was determined with a Phillipson microbomb calorimeter (Phillipson, 1964) and total organic nitrogen content (% dry weight) of plants, larvae, and feces was determined by the micro-Kjeldahl technique (McKenzie and Wallace, 1954).

Data were subjected to one-way analyses of variance, and differences between means were examined by Tukey's studentized range (Snedecor and Cochran, 1967) or a *t*-test. Correlation coefficients from linear regression analyses were tested for significance using a table of critical '*r*' values. The analyses were run on an Olivetti Underwood Programma 602.

Parameters of Larval Growth

From our data we calculated the following parameters of larval growth and feeding efficiency (Waldbauer, 1968; Kozlovsky, 1968):

G.R.: Growth Rate = mg (dry wt.) biomass gained/day

R.G.R.: Relative growth rate = mg biomass gained/mg larval biomass/day

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[1] Voucher specimens have been placed in the Cornell University Insect Collection (Lot 1023: Sublot 21)

C.R.: Consumption Rate = mg (dry wt.) food ingested/day

R.C.R.: Relative Consumption Rate = mg biomass eaten/mg larval biomass/day

A.D.: Approximate Digestibility
(also called Assimilation Efficiency)

$$=\frac{\text{Food ingested (mg dry wt.)} - \text{Feces (mg dry wt.)}}{\text{Food ingested (mg dry wt.)}} \times 100\%$$

E.C.D.: Efficiency of Conversion of Digested Food
(also called Net Growth Efficiency)

$$=\frac{\text{Biomass gained (mg dry wt.)}}{\text{Food ingested (mg dry wt.)} - \text{Feces (mg dry wt.)}} \times 100\%$$

E.C.I.: Efficiency of Conversion of Ingested food
(also called Gross Growth Efficiency)

$$=\frac{\text{Biomass gained (mg dry wt.)}}{\text{Food ingested (mg dry wt.)}} \times 100\%$$

E.C.I.: $= \text{AD} \times \text{ECD}$ (= Overall Efficiency)

The following terms are used to describe the utilization of nitrogen (see also Slansky and Feeny, 1977):

Relative N.A.R.: Relative Nitrogen Accumulation Rate = mg biomass nitrogen gained/mg larval biomass/day

Relative N.C.R.: Relative Nitrogen Consumption Rate = mg nitrogen ingested/mg larval biomass/day

N.U.E.: Nitrogen Utilization Efficiency $= \dfrac{\text{mg biomass nitrogen gained}}{\text{mg nitrogen ingested}} \times 100.$

Water Content of Excised (Experimental) Leaves

To estimate the average water content of the leaves fed to each group of experimental larvae, ten cherry leaves were exposed to each of four treatment conditions, identical in all respects to those used for the feeding experiments except that larvae were absent. From each treatment group, individual leaves were withdrawn at intervals to estimate changes in water content throughout 72 h (Fig. 1). Since the average undisturbed feeding period of the larvae (time between introduction of food and its removal) during the feeding experiment was 30 h, the change in water content of the leaves in each treatment after 15 h was considered the mean value. The change in leaf weight (as a percent of original weight) at the midpoint of the feeding interval was +13.5% for treatment 75+ and −4%, −10.0% and −20.0% for treatments 75−, 35+, and =35−, respectively (see Fig. 1). The mean leaf water contents for the 4 treatments (75+, 75−, 35+, 35−) were 69.9%, 58.9%, 55.4% and 49.1%, respectively, from a mean initial value of 61.5%.

Relative Maintenance Costs of Larvae

Calculation of "minimal" maintenance costs was based on larvae in treatment 1 (75%+) where the smallest respiratory expenditures occurred. To determine if larvae in drier treatments (75−, 35+, 35−) were metabolizing energy in excess of this basal "minimal" amount, I considered the average weight of the larvae during the feeding period, and also the duration of the period.

The total maintenance cost, in respired calories, was calculated for each treatment by subtracting the total calories actually incorporated in the larval body from the total calories assimilated (ingested calories minus fecal calories). Knowing the total energy expended, the relative maintenance cost (R.M.C.) was computed by dividing the total maintenance costs for the larvae in a given treatment by the product of the average dry weight of the larvae during that instar and the duration of

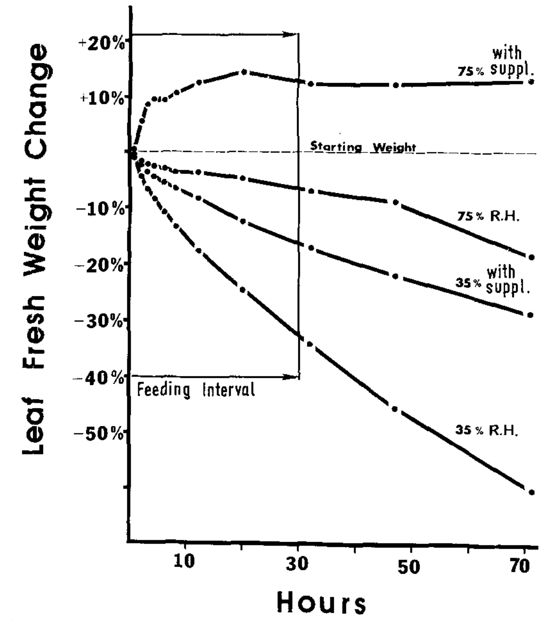

Fig. 1. Leaf water loss and gain as a function of time when experimentally subjected to two relative humidity regimes (each one with and without leaf water supplementation via the petiole; see "Methods and Materials")

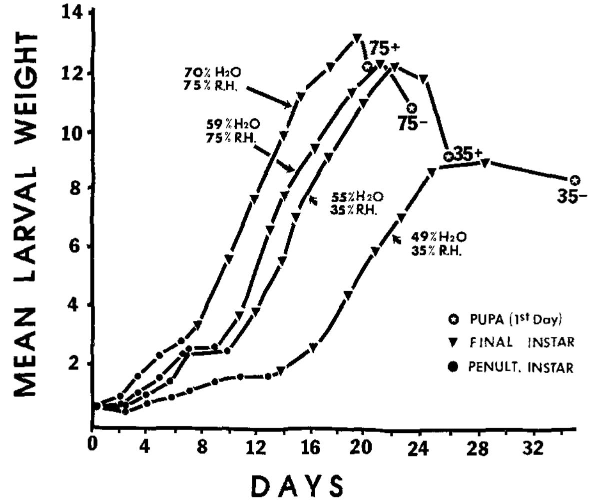

Fig. 2. Growth curves (mg fresh wt. as a function of time) for *Hyalophora cecropia* larvae on *Prunus serotina* from the penultimate (4th) instar through to pupation in four humidity and leaf water treatment regimes

the stadium in days. The average R.M.C.'s are represented as calories expended per milligram of larval dry weight per day. This R.M.C. for larvae in the 75%+ R.H. conditions was used to calculate the "expected minimal" costs for maintenance and larval growth in other treatments when multiplied by the product of the average dry weight of the larvae and the duration of the stadium for larvae in these other treatments. Comparisons of the actual calories expended in respiration with the predicted minimal (or basal) requirements were made for each treatment in order to assess the "additional" costs of growth for larvae lacking adequate water.

Seasonal Variation of Leaf Water Content

Six *P. serotina* trees, between 3 and 12 m tall, were sampled at monthly intervals during 1975. Each was at the edge of woodland or in a mature hedgerow within one mile of the Cornell University campus near Ithaca, New York.

Table 1. Larval growth rate and pupal weights of *H. cecropia* on *P. serotina* leaves under different conditions of relative humidity and leaf water supplementation[a]

Treatment	Duration of instar (4th) (days)	Duration of instar (5th) (days)	Pupal[b] weight (mg fresh)	Larval growth rate (5th) (mg fresh/day)
75%+	7.54a	12.31a	12,238.87a	808.1a
	0.54	0.66	1,091.82	53.0
75%−	9.37b	14.10a	11,718.85ab	656.5b
	0.26	0.38	362.32	18.7
35%+	10.27c	15.08a	9,215.06ab	556.2b
	0.47	0.52	762.57	14.1
35%−	13.02d	21.30b	8,820.60b	383.2c
	0.54	1.44	1,026.16	28.3
L.S.D.[c]	0.45	3.06	3,320.27	129.0
	*	*	*	*

[a] Data presented as a mean ± S.E.

[b] Fresh weight 1st day after completion of cocoon

[c] Asterisk denotes significant differences ($\alpha = 0.05$ level) via Tukey's 'studentized range' ($Q_{0.05}s_{\bar{x}}$), with those means differing significantly indicated by different letters

Table 2. Feeding performance (dry weight basis) of 4th instar *H. cecropia* larvae on *P. serotina* are presented as a mean ± S.E.)

Treatment	Total food consumed (mg dry wt.)	Total excreta produced (mg dry wt.)	Total weight gain/larva (mg dry wt.)	Dry weight consumption rates	
				(C.R.)[a]	(R.C.R.)[b]
75+	984.61	665.28	254.27	131.60	0.841
	77.19	48.19	23.97	9.84	0.080
75−	1137.00	782.35	286.36	121.39	0.746
	94.48	58.56	30.15	9.27	0.074
35+	1297.46	863.63	277.59	126.69	0.704
	87.14	73.12	19.69	7.17	0.054
35−	1429.25	900.34	179.42	109.68	0.804
	155.96	89.24	22.56	11.26	0.031
L.S.D.[c]	432.23	276.09	95.52	37.02	0.235
	*	n.s.	*	n.s.	n.s.

[a] C.R. = mg food/day (see "Methods and Materials")

[b] R.C.R. = mg food/mg larva/day (see "Methods and Materials")

[c] Asterisk denotes significant differences at $\alpha = 0.05$ via Tukey's 'studentized range' ($Q_{0.05}s_{\bar{x}}$)

On each sampling date two branches (one shaded and one exposed) were removed from each tree between 1100 and 1330 h and returned immediately to the laboratory; 3 tip and 3 base leaves from each branch were then selected randomly, immediately weighed, and then lyophilized to determine dry weight.

Variation of leaf water content in tree #1 was examined over two 48 h periods (June 24–26 and August 27–29). Base (mature) and tip (immature) leaves were sampled randomly throughout these 2-day intervals. The microclimate of this tree was monitored with a recording hygrothermograph housed next to the tree at mid height.

Results

Effects on Larvae

The growth rate of 4th and 5th instar cecropia larvae decreased with decreasing leaf-water (Fig. 2). The duration of these instars was increased, and the peak larval weights and mean pupal weights decreased progressively from treatment 75%+ through treatment 35%− (Table 1). In a feeding experiment undertaken during the 4th (penultimate) instar, larvae in treatment 35%− consumed more food, gained less weight, were less efficient at utilizing assimilated food (E.C.D.), and grew more slowly than larvae in treatments with more leaf water (Table 2).

No differences in nitrogen or caloric content in foodplant leaves between treatments were evident, though leaf-water content decreased significantly from the first through fourth treatment (Table 3). Poor larval performance and slower

leaves under different conditions of relative humidity and leaf water supplementation (all data

Efficiency (dry wt. budgets)			(Dry wt. basis) Growth rates	
Assimilation efficiency (A.D.)	Net efficiency of growth (E.C.D.)	Gross efficiency of growth (E.C.I.)	(G.R.) (mg/day)	(R.G.R.) (mg/mg/day)
32.09	81.52	25.70	33.83	0.216
2.39	5.08	0.47	2.62	0.018
30.94	81.20	24.94	30.61	0.188
1.29	3.93	0.82	3.10	0.023
33.52	67.10	21.49	27.09	0.150
3.02	7.00	1.05	1.64	0.009
36.61	34.04	12.45	13.80	0.100
0.83	0.89	0.39	1.66	0.004
7.99	19.35	2.94	9.04	0.058
n.s.	*	*	*	*

Table 3. Caloric and nitrogen contents of *Prunus serotina* leaves under different regimes of relative humidity and leaf water supplementation[a]

Treatment	Nitrogen[b] content (% dry wt.)	Caloric[b] value (per mg dry wt.)	Leaf-water[c] content (%)
75+	2.81	4.61	69.97a
	0.04	0.03	1.40
75−	2.75	4.71	58.95b
	0.13	0.08	1.18
35+	2.64	4.73	55.49c
	0.07	0.04	1.11
35−	2.69	4.64	49.12d
	0.02	0.08	0.98
Values of leaves at the time of introduction as fresh food	2.75	4.69	61.53
	0.07	0.03	1.93
L.S.D.[d]	0.44	0.26	1.93
	n.s.	n.s.	*

[a] Data are presented as a mean ± S.E.

[b] From analysis of leaves at 24 h

[c] Mean leaf water calculated from data in Figure 1 (see "Methods and Materials")

[d] Asterisk denotes significant differences via Tukey's test ($Q_{0.05} s_{\bar{x}}$) with means which are significantly different indicated by different letters

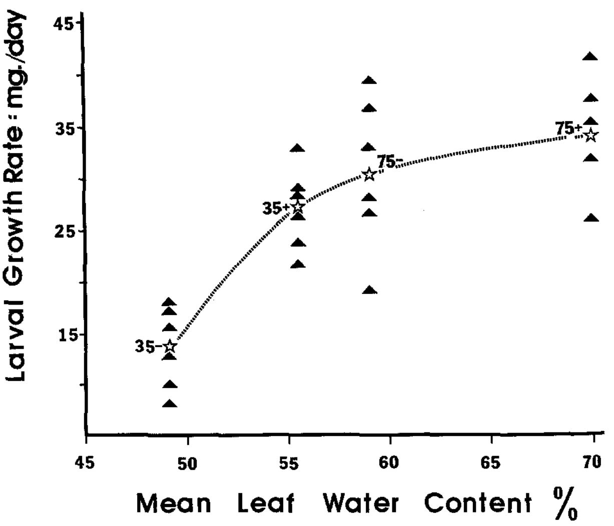

Fig. 3. Larval growth rate (mg dry wt. gained/day) of 4th instar *H. cecropia* larvae as a function of average leaf water content in each of 4 treatment regimes during the feeding experiments. Individual larval performances are presented with the mean for each treatment indicated by a star

larval growth were related to decreasing leaf-water content as affected by the R.H. and availability of water supplied via the petiole (Fig. 3).

Explanation of Effects

To understand how availability of leaf-water limited larval growth rate, I examined the components of growth rate individually and in detail. Growth rate = Consumption Rate × Assimilation Efficiency × Net Growth Efficiency (see "Methods and Materials").

Consumption rates of food-plant tissue did not differ significantly from one treatment to another (Table 2). Since biomass consumption rates were similar on a dry weight basis, they could not themselves account for the observed differences in growth rates between treatments. Since there were no significant differences in the assimilation of leaf biomass (= A.D., or digestibility), this could not be invoked as an explanation of the observed differences between treatments in larval growth either. The third component of growth rate, the efficiency of conversion of assimilated biomass into larval tissue (= E.C.D.) was significantly lower for treatment 35 − (Table 2), and this reduction appeared to be largely responsible for the decreased larval growth performance in this treatment.

Effects of Leaf Water upon Larval Energy Budgets

The energy conversion efficiencies (E.C.D. and E.C.I.) were suppressed nearly 3-fold in the treatment lowest in leaf water (Table 4). The metabolic cost, or total calories spent in respiration, for larvae in treatment 4 was nearly eight times greater than that for larvae in treatment 1 and the relative maintenance costs (calories expended/mg, biomass/day) were nearly five times as high (Table 4). The efficiency of assimilating calories was not affected by variations in leaf water content (Table 4).

Analysis of the composition of larvae from the four treatments (Table 5) indicates a reduction in stored calories and an increase in the proportion of nitrogen in larvae subjected to the drier treatments. This presumably resulted from the differential catabolism of larval carbohydrate and/or fat in preference to protein.

The residual, or unassimilated, energy in excreta (calories/mg) shows only a small reduction from that energy initially contained in the food-plant leaves. The energy extraction efficiencies (% decrease of energy from leaves to feces) in this experiment ranged from 2.1% to 5.5% and were not significantly different between any of the four treatments.

Effects of Leaf Water upon Utilization of Nitrogen

Efficiency of extraction (see Pandian and Delvi, 1973) of leaf nitrogen (41.8–65.1%) was greater than that of energy and was reflected in the relatively

Table 4. Caloric expenditures of 4th instar larvae of *H. cecropia* under different conditions of relative humidity and leaf water supplementation[a]

Treatment	Total calories assimilated	Total calories actually expended in respiration	"Predicted minimal" calories to be expended in maintenance respiration	Caloric Budget			Relative maintenance costs of larvae (R.M.C.) (calories expended/mg tissue/day)
				Assimilation efficiency (A.D.)	Conversion efficiency (E.C.D.)	Overall efficiency (E.C.I.)	
75+	1551.98a	206.96a	(206.96)[b]	33.86	88.43a	29.49a	0.178a
	198.94	118.36	–	2.33	5.15	0.54	0.088
75–	1862.71a	399.43a	280.74	34.55	78.78ab	27.06ab	0.248a
	197.45	80.97	30.54	1.28	3.56	0.89	0.040
35+	2311.20a	878.87a	335.47	37.74	64.33b	23.44bc	0.505ab
	209.03	225.22	28.09	2.83	6.19	1.15	0.161
35–	2499.13b	1593.10b	315.59	37.29	36.37c	13.56d	0.887b
	317.18	205.25	32.17	0.82	0.42	0.42	0.047
L.S.D.[c]	934.89	673.30	–	7.63	17.03	3.21	0.381
	*	*	–	n.s.	*	*	*

[a] Data are presented as a Mean ± S.E.

[b] This figure was used as a standard to compute the other predicted minimal maintenance costs relative to the average dry weight during the instar and duration of the stadium (see "Methods and Materials")

[c] Asterisk denotes significant differences at $\alpha=0.05$ via Tukey's 'studentized range' ($Q_{0.05}\, s_{\bar{x}}$), with means differing significantly indicated by different letters

Table 5. Nutritive content of 4th instar *H. cecropia* larvae and their excreta under different conditions of relative humidity and leaf-water supplementation (all data are presented as a mean ± S.E.)

Treatment	Relative humidity	Larvae[a]			Excreta[b]		
		Water content (%)	Calories (per mg dry wt.)	Nitrogen (% dry wt.)	Water content (%)	Calories (per mg dry wt.)	Nitrogen (% dry wt.)
75+	75%	88.68	5.29	8.43	54.40	4.49	0.96
		0.30	0.03	0.24	2.14	0.06	0.04
75–	75%	85.45	5.11	9.07	50.26	4.47	1.19
		0.90	0.01	0.10	1.50	0.06	0.02
35+	35%	86.09	5.16	9.03	44.10	4.43	1.10
		0.22	0.06	0.21	1.40	0.03	0.01
35–	35%	83.78	5.05	10.36	27.05	4.59	1.60
		0.34	0.04	0.26	1.54	0.04	0.02
A fresh sample at start of feeding experiment		88.25	5.24	8.45	–	–	–
		0.51	0.04	0.15			
L.S.D.[c]		2.37	0.19	1.02	6.66	0.22	0.11
		*	*	*	*	n.s.	*

[a] Samples of 4th instar larvae were taken towards the later part of the stadia

[b] Analysis of feces (collected daily throughout the stadia)

[c] Asterisk denotes significant differences at $\alpha=0.05$ via Tukey's 'studentized range' ($Q_{0.05}\, s_{\bar{x}}$)

Table 6. Utilization of nitrogen by 4th instar *H. cecropia* larvae fed on *P. serotina* leaves under different conditions of leaf-water availability (Ithaca, N.Y.)

Treatment[a]	Total N ingested (mg dry)	N.C.R.[b] (mg N/mg biomass/day)	N.U.E.[c] (%)	N.A.R.[d] (mg N/mg biomass/day)
75+	27.77	0.0228	76.84a	0.0176a
	2.18	0.0018	1.42	0.0017
75−	31.27	0.0200	82.29a	0.0166a
	2.60	0.0017	2.70	0.0017
35+	34.38	0.0181	73.32a	0.0132ab
	2.31	0.0010	3.59	0.0009
35−	38.45	0.0216	47.99b	0.0104b
	4.20	0.0023	1.50	0.0013
L.S.D.[e]	11.64	0.0051	9.90	0.0051
	n.s.	n.s.	*	*

[a] Data are presented as a mean ±S.E. Treatment conditions are described in "Methods and Materials" (+indicates water supplementation via leaf petiole)

[b] Relative nitrogen consumption rate (N.C.R.)

[c] Nitrogen utilization efficiency (N.U.E.) = mg N assimilated/mg N ingested × 100%

[d] Relative nitrogen accumulation rate (N.A.R.)

[e] Least significant differences required at $P=0.05$ (Tukey's studentized range test) are denoted by an asterisk and indicated by different letters

depleted fecal nitrogen (Table 5) relative to leaf nitrogen content (Table 3). Water content of feces was significantly reduced in drier treatments, suggesting more efficient extraction by larvae in these treatments. However, these values can not be used directly for calculation of a water budget since the feces may have dried differentially after egestion.

There was a significant reduction in the fecal nitrogen of larvae fed leaves high in leaf water relative to those of treatment 4 (Table 5). Nitrogen budgets, calculated for each treatment, indicated that although the nitrogen consumption rate (mg nitrogen/mg larval biomass/day) was identical in all treatments, the efficiency of nitrogen assimilation (nitrogen utilization efficiency: N.U.E.) was poorer in treatments with low leaf water (Table 6).

The Nitrogen Accumulation Rate (N.A.R.) was significantly suppressed in larvae subjected to the low water treatment (Table 6). Larval growth rates in the four treatments were highly correlated with larval nitrogen accumulation rates ($r=0.992$), emphasizing the importance of nitrogen in relation to available water in the growth in this herbivore.

The cherry leaves used for the feeding experiment averaged 2.6–2.8% total nitrogen by dry weight (Table 3). This corresponds to the period in early August when larvae typically reach the fourth instar. The interaction between leaf water content and larval utilization of nitrogen may differ at other stages of growth since the concentration of total nitrogen in leaves decreases throughout the season. The nitrogen content of leaves on tree #2 decreased from 5.3% in mid-May to 3.9% in early June, 3.0% in mid-July, 2.4% in mid-August, and

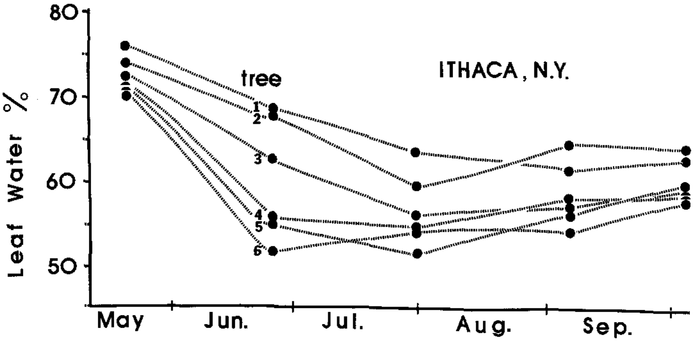

Fig. 4. Seasonal variation of leaf water content in six *Prunus serotina* trees near Ithaca, N.Y. For each tree (numbered 1 through 6) values are means from 12 leaves on each sampling date (see "Methods and Materials" for details)

Table 7. Seasonal leaf-water content of two *P. serotina* trees in Ithaca, N.Y. (1975). All samples were taken between 11 a.m. and 1:30 p.m.[a]

	Orientation of leaves	Location on stem	June 2 100% R.H.	June 25 72% R.H.	July 29 80% R.H.	Sept. 5 100% R.H.	Oct. 4 73% R.H.	Oct. 30 75% R.H.[b] (grouped by color)
Tree 1	Sun	tip	74.09	71.81	64.98	61.36	63.08	66.34g-r
			2.27	0.23	0.83	3.73	0.38	0.37
		base	67.99	66.61	61.07	60.29	62.88	67.88r-y
			1.03	0.25	1.04	0.57	0.75	1.30
	Shade	tip	78.64	68.49	64.39	61.35	60.56	48.72y-b
			0.32	0.62	0.42	0.65	0.58	2.16
		base	72.53	66.65	63.52	62.35	64.83	
			0.73	0.37	0.27	0.73	3.16	
Tree 2	Sun	tip	78.67	67.66	55.90	61.02	59.54	68.69r-y
			0.20	2.06	0.14	0.20	1.47	1.95
		base	71.27	64.84	56.53	62.11	59.47	51.31y-b
			0.61	1.85	0.24	0.85	0.73	4.55
	Shade	tip	78.77	72.08	64.94	68.03	66.71 (sen)	26.34b
			0.52	0.32	0.92	0.67	0.95	1.90
		base	71.98	66.54	61,24	69.77	70.84 (sen)	
			0.48	0.28	2.07	1.61	1.51	
	Mean			68.09	61.57	63.29	63.49	–
	(±S.E.)			0.92	1.29	1.25	1.38	
tip vs. base[c]			***	**	n.s.	n.s.	n.s.	–
sun vs. shade[c]			n.s.	n.s.	n.s.	*	n.s.	–

[a] All values are presented as a mean ±S.E.

[b] October 30 leaves are grouped by color and not by position on the free (color code of senescing leaves: g = green, r = red, y = yellow, b = brown)

[c] Asterisk denotes significant differences via students' *t*-test (* = 0.10 level, ** = 0.05 level, *** = 0.001 level)

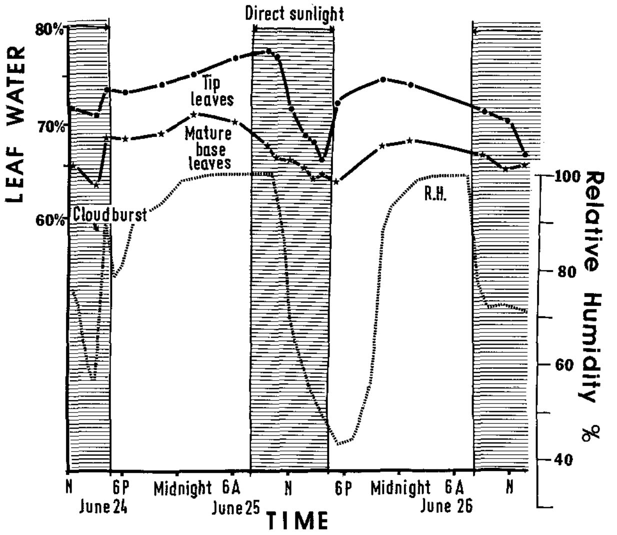

Fig. 5. Variation in leaf water content of *P. serotina* (tree #1) from June 24-June 26 in 1975. Tip and basal leaves on a stem were sampled separately (4 to 6 leaves at each sampling time) (see "Methods and Materials")

2.1% in mid-September. At the end of October, during abscission-layer formation, the nitrogen dropped to 1.5% for reddish-green leaves and then to 0.7% for yellow-brown leaves.

Natural Variation of Leaf Water in the Field

Within a single host-plant species, *P. serotina,* the leaf water content varied both seasonally and also between different individual trees on any given date (Fig. 4). The mean leaf water content on six sample trees on 25 June, for example, ranged from approximately 50% to 70% (compare Table 3). A more detailed description of seasonal leaf-water variation upon two representative individual trees is presented in Table 7. Except for the September 5th sampling date, when a heavy dew (R.H. = 100%) was still in the process of evaporating under direct sunlight, there were no significant differences in water content between sun and shade leaves. There were no significant differences between tip and base leaves except for the first sampling date in June. At this early summer sampling date obvious differences in leaf maturity were evident between the fully expanded base leaves and the younger, succulent, half-grown leaves at the tips of the branches.

Daily variation in relative humidity accounted for a considerable portion of the leaf-water variation (Fig. 5). Leaf-water content rose rapidly after the June 24th rain and gradually at night. Water content of younger (June) leaves was more responsive to variations in relative humidity than that of mature leaves. Relative humidity for the Ithaca summer of 1975 ranged from 30%

to 100%, with typical daily ranges of 40% to 100%. The environmental conditions chosen for the feeding experiments lie well within the natural range of variation in leaf-water content and relative humidity experienced by larvae in the field.

Discussion

Terrestrial arthropods have evolved a variety of adaptations which reduce their susceptibility to desiccation and increase their ability to acquire adequate nutritional water (see reviews by Buck, 1953; Edney, 1957; Craig, 1960; Beament, 1961, 1964; Barton-Browne, 1964; Wigglesworth, 1965; Berridge, 1970; Wharton and Arlian, 1972; Candy and Kilby, 1975; Cloudsley-Thompson, 1962, 1964, 1975). Most important among these adaptations are the impermeable epicuticle and control of the opening and closing of the spiracles during respiration. Physiological regulation of reabsorption of water via the Malpighian tubules, free water uptake, metabolic water production and behavioral phenomena such as photonegativity, burrowing, leaf-mining, leaf-rolling, or spittlemass-dwelling also contribute to survival in desiccating terrestrial environments.

In spite of adaptation, however, *H. cecropia* larvae evidently encounter climatic conditions in which their growth is limited by low leaf water content and relative humidity. Although most environmental water vapor activities favor a net loss of water from an animal's water pool (Arlian, 1975), tree leaves low in water content imposed especially slow growth and additional metabolic costs upon larvae which used them for food. Large amounts of leaf cellulose fiber (Soo Hoo and Fraenkel, 1966; Fogal, 1974) and tannins (Feeny, 1970), associated with low nitrogen content probably contribute to the slow growth of many lepidopterous larvae feeding on mature tree leaves (Scriber, 1975; Feeny, 1975, 1976; Scriber and Feeny, in preparation). The results of the present study emphasize the additional role which may be played by low leaf water content.

These experiments essentially controlled for the differences in the relative amounts of protein, fiber, and calories of one foodplant. This permitted the investigation of the role of leaf water itself as a cause of the reduced net efficiencies of biomass conversion (E.C.D.) reported in Feeny (1975) on plants with low water content (i.e., trees). Physiological responses of cecropia larvae to low water could include the following: faster feeding, a greater efficiency of water extraction, use of free drinking water, or the production of metabolic water. I have found that consumption rates were not increased by larvae in low water treatments as would have been expected if digestive efficiency was being sacrificed to maximize rate of passing food through the hindgut for increased water extraction. It did seem as though larvae compensated for low concentrations of water by increasing the efficiency of water extraction from food passing through the gut. This method was apparently not completely adequate, as those larvae in treatments with lower leaf water continued to desiccate. Providing drinking water by misting transplanted trees several times daily in the same four treatment conditions had little effect on larval growth relative to leaf-water content (Scriber, 1975). Larvae in drier treatments expended increasing portions of assimilated energy beyond the calculated basal requirements, and these higher relative maintenance costs (calories expended/mg biomass/day) were not due to increased consumption rates or increased costs of conversion of plant biomass between treatments. They may instead be directly related to the necessary production of metabolic water.

Metabolic water production has been shown to be important in the survival of certain larger animals (Schmidt-Nielson, 1975) and possibly for some insects (Fraenkel and Blewett, 1944; Arlian and Eckstrand, 1975). For other insects, metabolic water has been shown to be insufficient for growth and reproduction (Edney, 1966) or insignificant (Kanungo, 1965). Its production can sometimes even be deleterious, in that there can actually be a net loss of water during its production by the insect (Mellanby and French, 1958; Murray, 1968). For cecropia larvae, respiratory costs in excess of the calculated basal level required for growth may have resulted either from the production of metabolic water or from attempts at more efficient extraction of nitrogen and/or water.

Why does low water result in slow larval growth? The means by which larval growth was actually suppressed in drier treatments appears to be related to poor utilization (or assimilation) of nitrogen. This reduction in the efficiency of assimilating nitrogen resulted in lowered relative nitrogen accumulation rates (N.A.R.'s) and correspondingly slow growth. Although total leaf nitrogen was not a differential factor in the four treatments of this experiment, it may be an important regulatory factor seasonally in the field. Seasonal decrease in leaf nitrogen occurs for many species of plants (McHargue and Roy, 1932) and its importance to growth rates of leaf feeding insects is discussed elsewhere (Feeny, 1970; Slansky and Feeny, 1977). The seasonal variation in trace and major elements is also well documented for several plant species (McHargue and Roy, 1932; Tamm, 1951; Guha and Mitchell, 1966; Smirnov and Demenova, 1972; Woodwell, 1974; Woodwell et al., 1975) and may also be of significance to the growth rates of certain herbivores throughout the growing season.

In herbaceous plants of the Cruciferae family, where leaf water was less likely to be a limiting factor than with tree leaves, the contribution of plant nitrogen concentration and secondary chemicals upon *Pieris rapae* larval growth rates were more easily discerned (Slansky and Feeny, 1977). Although the rate of accumulation of nitrogen was optimized for *P. rapae* larvae on various crucifers, this was achieved by high feeding rates on low nitrogen plants and low feeding rates on high nitrogen plants (Slansky, 1974a). For tree feeders, where leaf water may limit the nitrogen assimilation efficiency and nitrogen accumulation rate, a greater consumption rate would not contribute to faster growth. An increased rate of feeding on tree leaves would require greater energy expenditures for the larvae, and would contribute little toward maintaining an adequate proportion of body water. In fact, a survey of 22 species of lepidoptera on over 100 species of natural food-plants indicate that tree leaf feeders in general have significantly lower consumption rates (mg food consumed/mg larval biomass/day) than shrub or herb feeders (Scriber, 1975). A recent review of the physiological mechanisms and environmental factors regulating the intake of food and water by insects is provided by Barton-Brown (1975).

The figure which appears in Feeny (1975) relates to the fact that leaves of trees and shrubs are relatively low in water compared to herbs or forbs. "Apparent" or "predictable" (Feeny, 1976; Rhoades and Cates, 1976) plant resources such as mature tree leaves appear to be defended by "quantitative" defenses (Feeny, 1975) including tannins, fiber, silica, lignins, or resins which may reduce the digestibility of plant tissues, thereby lowering the growth rate and presumably the fitness of herbivores. This experiment suggests that low water concentrations may also be part of the defenses of apparent plants.

Slow growth contributes to the apparency of tree leaf feeders, and may have been a principal factor influencing the ecological strategies or adaptive syndromes of tree feeders as a group relative to herb feeders (Slansky, 1974b; Feeny, 1976). Early spring feeding on oaks enabling larvae to utilize tree foliage that is less tough, less quantitatively fortified with tannins, and nutritionally more favorable is another ecological alternative (Feeny, 1970) which more resembles a strategy of early successional or herb feeding larvae. Ephemoral plant tissues appear to rely less upon the large quantities of chemical defenses used by apparent, or predictable, plants (those which can not "escape in space or time"), and more upon a diversity of "qualitative" (see Feeny, 1975) toxins or repellants (Cates and Orians, 1975; Feeny, 1976; Futuyma, 1976; Rhoades and Cates, 1976).

In addition to revealing an important interaction between leaf water and larval growth rates, this experiment emphasizes the need for caution in the interpretation of laboratory feeding experiments in which leaf water content is not adequately maintained, either by high atmospheric humidity or else by supplementation via the petiole. Both efficiency and rate of conversion of plant biomass into larval biomass can be severely limited unless leaf water content is maximized. This limiting effect of low leaf water content on larval growth performance would presumably be more pronounced in mature tree leaves with 50–65% water than for herb or forb leaves with 85–95% water.

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