Effect of Leaf Age and Photoperiod on Buck Moth (Lepidoptera: Saturniidae) Larval and Pupal Development[1]

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ABSTRACT Dietary "early season" live oak foliage and longer scotophase accelerated larval development and resulted in heavier pupae of the buck moth, *Hemileuca maia* (Drury). Female pupae were heavier than male pupae under all conditions. Males required significantly fewer days to pupate. Rearing at a shortened scotophase on more mature foliage reduced larval survival. Phenolics and tannins were higher in early season oak foliage than in late spring foliage.

KEY WORDS Buck moth, *Hemileuca maia,* development, live oak, tannins, photoperiod.

The buck moth, *Hemileuca maia* (Drury), is a univoltine species that occurs from Nova Scotia to the Gulf Coast and west to the Great Plains (Holland 1968). The length of developmental stages of *H. maia* is variable by geographic region, but development includes overwintering as eggs and a summer pupal diapause (Holland 1968, Tuskes 1984).

In southern Louisiana, eggs hatch in late March and larvae feed gregariously on the preferred host, live oak (*Quercus virginianus* Mill). The larvae normally complete six instars and pupate beneath 3-5 cm soil during late May or early June. Adults emerge in late November or early December and females lay their eggs on distal branches of the host plant.

Our interest in this insect focuses on its univoltine life cycle and the factors responsible for modulating this life history strategy, particularly its utilization of seasonal oak foliage and its lengthy summer pupal diapause. The influence of several environmental factors known to affect summer diapause, including host plant quality and photoperiod (Masaki 1980), were examined in the present study. Also, because of similarities in buck moth life history to that of the winter moth, *Operophtera brumata* L. (Feeny 1970), the levels of tannins in leaves fed to larvae were monitored.

Methods and Materials

Insects, Host Foliage and Photoperiod

The effect of gender on larval and pupal duration was examined. Two larval groups, each from an individual egg mass, were field collected on 26 March 1984 and maintained separately in the laboratory until adult eclosion. Larvae were

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reared on fresh live oak foliage at 27 ± 0.5°C and LD 12:12. Twigs with leaves intact were rinsed with deionized water. Prior to feeding, twigs were placed in vials containing water to prevent premature leaf desiccation. Pupae were held at 21 ± 0.5°C, LD 12:12 until eclosion. Sixth instar duration and pupal duration were recorded for each individual.

Larvae from ten egg masses were collected from the field from 27-30 March, 1981 and were maintained in the laboratory on fresh oak foliage. On April 7, all larvae were pooled, randomly separated into four groups of 110-118 larvae, and placed into 30.5 × 30.5 × 30.5 cm screened cages. Each larval group received one of the following treatments: LD 10:14 and "early" leaves (leaves collected between March 30 and April 3 and stored at -20°C), LD 14:10 and "early" leaves, LD 10:14 and "weekly" leaves (leaves collected weekly from March 27 to May 22 and stored at -20°C at least 24 h prior to feeding), and LD 14:10 and "weekly" leaves. Two additional groups of 20 and 23 larvae were held in 4-liter cardboard containers at LD 10:14 or LD 14:10, respectively, and were fed only freshly collected leaves until pupation, serving as controls for frozen foliage effects. All larvae were maintained at 24 ± 0.5°C and 70-80% relative humidity until pupation. Larval survival to pupation and larval duration were recorded. Ten or 15 pupae from each treatment (excluding controls) were placed in the field and monitored for adult eclosion. The remainder were used in a separate study (Foil et al., in prep.).

Tannin Assays. Leaves of approximately equal size were selected from nine weekly leaf samples collected in 1981 plus an additional sample taken 29 September 1981 and were lyophilized, ground to a coarse powder, and extracted in aqueous methanol to obtain tannins (Martin and Martin 1982). Total extractable phenolic content was determined using the Folin-Denis assay (Martin and Martin 1982). Four aliquots from each extract representing 0.06, 0.12, 0.18, and 0.24 mg equivalents of dried oak leaves provided data points to define a regression of dry leaf weight on total extractable phenolic content. Phenolic content was measured spectrophotometrically as absorbance at 725 nm (A_{725}).

An additional set of five aliquots representing 1.0-3.6 mg equivalents of dried oak leaves was used in a modification of the protein precipitation tannin assay of Hagerman and Butler (1978). D-Ribulose 1,5-biphosphate carboxylase/oxygenase (RuBPC) (Sigma Chemical Co., St. Louis, MO), an abundant protein in most plant foliage, was used in place of bovine serum albumin (Martin and Martin 1983). The protein-tannin precipitate formed was washed and then dissolved in 3 ml of 1% sodium dodecyl sulfate (SDS) containing 5% triethanolamine (v/v). One ml of a 0.01 M $FeCl_3$ in 0.01N HCl solution was added and a regression of dry leaf weight on precipitating tannin (measured spectrophotometrically as absorbance at 510 nm [A_{510}]) was determined. In addition, four aliquots of each extract representing 0.24, 0.48, 0.72, and 0.96 mg equivalents of dried oak leaves were added to SDS buffer-$FeCl_3$ reagent, and a regression of dry leaf weight on tannin concentration (A_{510}) was determined (Hagerman and Butler 1978). This assay allowed a relative comparison of total tannins in the plant extracts with tannins that precipitated protein. Three to six replicates of each tannin assay method were performed on each weekly oak leaf sample.

Statistical Analysis. The data for the dependent variables 6th instar duration, pupal weight and the interactions of larval duration, foliage type and photoperiod were strongly departed from normal distribution. Thus the data were ranked and appropriate analyses were performed on the ranked data (Conover and Iman,

1981). Larval and pupal duration in the gender study and the foliage/photoperiod study, and pupal weights were compared using median values at the 50% level obtained through the univariate procedure of SAS (SAS Institute, 1989). These values were ranked then compared using analysis of variance (ANOVA) (SAS Institute, 1989). Sixth instar duration data were not normally distributed and were analyzed with the ANOVA of Ranks procedure of SAS. Significance values for pupal duration and foliage/photoperiod larval duration data were assigned using Bonferroni's method (SAS Institute, 1989) with adjustments made to the larval duration data as suggested by Milliken and Johnson (1984) for a missing cell.

Larval survival among treatments was compared using a 3 × 2 factorial design which included photoperiod (two levels, LD 10:14 and LD 14:10), foliar age (two levels, early *vs* weekly/control) and freezing effects on foliage (two levels, control *vs* early/weekly). The survival data was frequency data and thus analyzed by the categorical data modeling procedure (CATMOD) of SAS (SAS Institute, 1989). Sample size for pupal survival was too small for categorical modeling, and the data were compared using Fisher's Exact Test (SAS Institute, 1989).

Data on pupal duration and leaf tannin content levels were normally distributed and analysis was performed on the raw data. For comparison of the tannin levels within leaves from the different sample dates, the simultaneous test procedure (Sokal and Rohlf, 1969) was used for each assay type to compare regression coefficients (Martin and Martin, 1982).

Results

Familial group and gender affected sixth instar duration. In a 2 × 2 factorial analysis of variance for sixth instar duration, both of the main effects (familial group and gender) were significant ($p = 0.0001$) with no significant interaction ($p = 0.3560$). Familial group B had a significantly longer sixth instar duration than familial group A; females had significantly longer sixth instar duration than males (Table 1). Main and interaction effects were not significant for pupal duration: $p = 0.4169$, $p = 0.7655$, $p = 0.1827$ for main effects of familial group, gender, or gender and group interaction, respectively. When mean sixth instar duration and pupal duration were summed, mean eclosion date for all groups was within a four-day period (Table 1).

Table 1. *Hemileuca maia* stage durations (in days) at 21°C, LD 12:12.

Familial Group	Sex	N	Sixth instar duration* (median value)	N	Pupal duration† (mean ± SEM)
A	M	51	19	37	168.2 ± 1.2
	F	32	21	29	165.7 ± 1.5
B	M	25	20	24	167.4 ± 1.7
	F	27	22	24	169.0 ± 1.8

* Medians were ranked then compared using the ANOVA of Ranks procedure of SAS. Duration of female instar was longer than male instar duration in both familial groups, and Group B instar duration was longer than that of Group A ($p = 0.05$).

† Mean pupal duration was not significantly different between gender or familial group as determined by the ANOVA procedure of SAS ($p = 0.05$).

There was a significant interaction of foliage age and photoperiod for larval survival ($p = 0.0052$) (Table 2). For simple main effects (i.e., testing for foliage age effects within each photoperiod, and for photoperiod effects within each foliage age) at both photoperiods, survival within both "early" and "control" foliage was significantly greater than "weekly" ($p = 0.0000$ and $p = 0.0363$, respectively at 10:14, $p = 0.0000$ for both at 14:10). Survival in "early" vs. "control" was not significantly different at either 10:14 ($p = 0.5667$) or 14:10 ($p = 0.1080$). For the simple main effect of photoperiod, survival at 10:14 was significantly greater than at 14:10 for "early" and "weekly" foliage, but not for "control" ($p = 0.0000$, $p = 0.0000$, $p = 0.5304$, respectively). None of the larvae reared on weekly leaves LD 14:10 survived. There were significant differences ($p = 0.0001$) for larval duration among the five observed treatment combinations reported in Table 2. The longer photophase (LD 14:10) prolonged larval development in all groups. Larvae fed early leaves had the most rapid development. Larvae reared on early leaves at LD 10:14 completed development earlier than larvae reared under any other foliage/photoperiod combinations. Larvae fed only freshly collected leaves throughout development (controls) took significantly longer to pupate at LD 14:10 than did larvae at all other treaments.

Host foliage age and photoperiod both influenced pupal weights ($p = 0.0001$) for both males and females (Table 3). Female pupae were heavier than male pupae within each treatment. Larvae reared on early oak foliage produced heavier pupae than did larval LD 14:10 controls for both sexes, and LD 10:14 control pupae were heavier than pupae from weekly foliage treatments. Longer scotophase resulted in heavier pupae for both males and females in the control treatments but had no effect on the early foliage treatment pupal weights. Pupal survival did not differ significantly among treatments (Table 3). There were significant differences among the four treatment combinations ($p = 0.0138$) for pupal duration (Table 3).

Table 2. The effects of live oak foliage age and photoperiod on *Hemileuca maia* larval development.

	Larvae			
Treatment	N	% Surviving*	N	Median Duration (days) †
Early LD 10:14	118	92	114	46 a
Early LD 14:10	111	64	76	52 b
Weekly LD 10:14	114	63	75	53 bc
Weekly LD 14:10	110	0	-	-
Control 10:14	20	90	18	54 c
Control 14:10	23	83	19	58 d

* Survival was compared using a 3 × 2 factorial design and the categorical data modeling procedure (CATMOD) of SAS. Early and control treatments were not significantly different; both were higher than the weekly LD 10:14 treatment ($p = 0.05$).

† Medians were ranked then compared using one-way ANOVA and were grouped using Bonferroni's Method ($p = 0.05$).

Table 3. The effect of live oak foliage age and photoperiod on *Hemileuca maia* pupal development. Larvae were maintained at 24°C and pupae were placed in field cages.

	Pupae				
	Median Weight (grams)*				Mean ± SEM
Treatment	Male (N)	Female (N)	N	% Surviving‡	Duration (days)†
Early LD 10:14	1.26 (51) a	1.91 (57) a	15	73 a	197.1 ± 1.2 a
Early LD 14:10	1.22 (38) a	1.59 (33) ab	10	60 a	190.7 ± 1.5 b
Weekly LD 10:14	1.06 (40) b	1.20 (32) c	10	60 a	196.0 ± 2.3 ab
Control 10:14	1.19 (6) a	1.44 (13) b			
Control 14:10	0.79 (9) b	0.93 (9) d			
Field Collected	-	-	10	80 a	197.2 ± 0.7 a

* Medians were ranked then compared using one-way ANOVA and were grouped using Bonferroni's Method ($p = 0.05$).

† Survival was compared using Fisher's Exact Test ($p = 0.05$).

Results of tannin assays on weekly oak foliage collections are presented in Fig. 1. Total phenolic concentration is not shown and, although values were higher, the seasonal trend was similar to that of total tannin (unpubl. data). Tannin concentrations are reported as regression coefficients (slopes) of A_{510} (tannins) *vs* mg dry leaf extracted. Higher slope values represent relatively higher tannin content as measured by spectrophotometric absorbance.

Total phenolic and tannin content was highest in early season leaves, dropped significantly during April, and returned to levels approaching those in early leaves by mid-May. The single September sample had a phenolic content similar to that measured for leaves collected in May.

The ratio of the regression coefficients of total tannins to protein-precipitating tannins was variable throughout the season (Fig. 1). The protein-precipitating tannins constituted 40-58% of the total tannins measured; a correlation coefficient of $r = 0.674$ was determined for the relationship of changes in protein-precipitating tannins on total tannins for all leaf samples combined.

Discussion

The sixth instar duration data (Table 1) indicate that females require slightly longer to reach the pupal stage than males. This difference probably reflects a lower weight requirement for male pupation. Differences in sixth instar duration between sibling groups, although only one day, were significant. Either a genetic component in larval development or synchronous development within groups could have influenced these results. Collecting data more frequently than once every 24 h would be required to address this phenomenon. However, these factors do not appear to influence the occurrence of male and female adult ecolosion. Adult eclosion is asynchronous in Louisiana, spanning a period of nearly one month.

Longer scotophase (LD 10:14) resulted in accelerated development for larvae fed early leaves compared with all other larval groups (Table 2). Photoperiod has been shown to alter larval development in other Lepidoptera. Shortened photophase accelerated development in *Dasychira pudibunda* L., a univoltine moth with

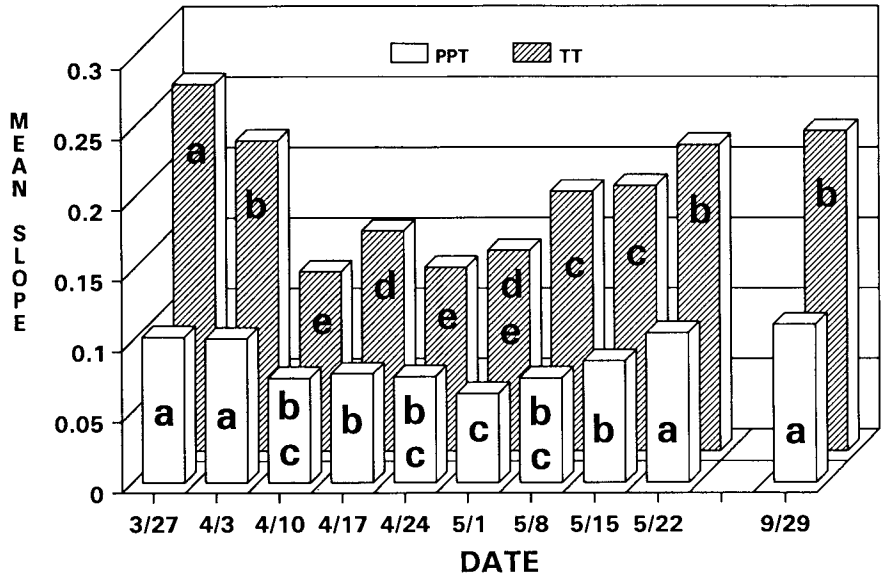

Fig. 1. Relative changes in tannin content of live oak, *Quercus virginianus*, leaves collected weekly from March 27 to May 22 and on September 29. Bars represent mean regression coefficients (slopes) of 3-6 replications. Bars bearing the same letter within an assay are not significantly different at $P < 0.05$, simultaneous test procedure, Sokal and Rohlf 1969. PPT = protein-precipitating tannin, TT = total tannin.

a winter pupal diapause (Geyspitz and Zarankina 1963). Growth rate was faster in *D. pudibunda* larvae held under shortened photophase and fewer larval instars were required to reach pupation. Longer scotophase also provides extended larval feeding periods for nocturnal feeders (Beck 1980). Although feeding behavior was not recorded in our study, larval development (Table 2) and pupal weights (Table 3) suggest that extended scotophase may favor buck moth larval feeding.

Larvae held in shortened scotophase and reared on early leaves also showed accelerated development compared with control larval (Table 2). Some factors that could have influenced these results include leaf toughness, water content, nutritional quality, and xenobiotics. Early leaves had the highest tannin content of any leaves in the study (Fig. 1). Lawson et al. (1984) examined total phenol and protein-precipitating tannin content of early- and late-season foliage from six oak species and found that young leaves had phenol and tannin levels at least as high as those of mature leaves. These compounds were reported to have no effect on nutritional indices measured for selected geometrid and saturniid larvae feeding on oak foliage (Lawson et al. 1984). Janzen and Waterman (1984) also found no significant seasonal changes in phenolic or tannin levels between young and mature leaves. Tree-feeding tropical saturniids, however, appeared to prefer host species rich in phenolics and condensed tannins (Janzen and Waterman 1984). The range caterpillar, *Hemileuca oliviae* Cockerell, a saturniid that feeds on grasses low in condensed tannin, showed no differences in survival or early instar development

between larvae fed control and low (0.01 and 0.1%) condensed tannin diets (Roehrig and Capinera 1983). High (1.0 and 10.0%) tannin diets were detrimental to development. Shorter stadia for larvae fed on 0.1 or 1.0% tannin diets were observed in some cases.

Differences in larval survival (Table 2) and pupal weights (Table 3) between LD 10:14 groups reared on control (fresh) and weekly (previously frozen) foliage suggest that freezing may have had a negative impact on leaf quality or acceptability, especially of mature leaves, since early leaves also had been frozen. Observations of early instar feeding indicated that freezing enhanced leaf toughness and desiccation in weekly leaves to a greater degree than in early leaves, making feeding on weekly leaves more difficult. Growth and development of weekly LD 14:10 larvae was delayed to such an extent that the larvae were unable to feed successfully on maturing foliage, resulting in total mortality in this group (Table 2). Neonate larvae offered previously frozen, late-season foliage collected in 1981 were unable to feed on the tough mature tissue (unpubl. data). Therefore, our data support the suggestion of Lawson et al. (1984) that the prudent spring feeding strategy of oak foliage feeders is one that favors rate over efficiency and the observation of Feeny (1970) that leaf toughness is a factor preventing late-season larval feeding.

The shortened pupal duration for larvae reared on early season leaves at LD 14:10 indicates that photoperiod during larval development, but not diet, influenced the pupal period (Table 3). Small sample sizes and lack of survival of weekly LD: 14:10 larvae preclude extensive interpretation of this experiment, and further work on this aspect of development appears warranted. However, our results are similar to those of Mansignh and Smallman (1967), who were able to shorten and even prevent winter pupal diapause in two saturniids by rearing larvae under long photoperiods.

The univoltine life cycle of *H. maia* appears to be well suited to seasonal changes in the foliage of its primary host, live oak. Egg hatch is synchronous with the addition of new foliage in this broadleaf evergreen, providing early larval instars with tender, highly nutritious leaves. As larvae mature, they become better able to handle unfavorable changes that often accompany leaf maturation, such as declining nitrogen and water content and increasing leaf toughness (Feeny 1970, Scriber and Slansky 1981). Buck moth larvae reared exclusively on early season oak foliage exhibited shortened larval duration and produced heavier pupae compared with larvae reared on maturing foliage. These data support studies indicating that young, tender foliage, typically high in nutritional nitrogen (Mattson 1980) and water (Scriber and Slansky 1981, Lawson et al. 1984), is a more efficient food source than mature foliage for this early-season feeder.

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