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DIFFERENTIAL GROWTH AND UTILIZATION OF THREE FOODPLANTS BY FIRST INSTAR LARVAE OF *CITHERONIA REGALIS* (SATURNIIDAE)

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ABSTRACT. One hundred larvae of *Citheronia regalis* (Saturniidae) were reared singly in petri dishes through the first instar on three different foodplants. They grew most rapidly on persimmon (*Diospyros virginiana*), less so on sweetgum (*Liquidambar styraciflua*), and most slowly on wing-rib sumac (*Rhus copallina*). Survival was high on persimmon and sweetgum, but on sumac it was only 67 percent, with many larvae refusing to feed, and others dying after ingesting some food. Frass pellet counts done on 12 larvae indicated that persimmon was the most advantageous plant for larval growth, with sweetgum being of intermediate food value. Sumac may contain repellent or toxic chemicals which adversely affect larval growth. The differences in foodplant suitability apparently are genetic in this moth strain. These results further support the theory that neotropical ebonies (*Diospyros*) and *C. regalis* represent a co-evolved plant-herbivore relationship of long-standing, with the more temperate plant genera (*Liquidambar* and *Rhus*) having been exploited as alternate food sources only in comparatively recent times.

Among insects herbivory is widespread, with most species accepting a limited range of foodplants and exhibiting preferences for only one or two of these (Matthews & Matthews, 1978). Feeding responses are elicited by chemo-sensory cues (Dethier, 1966, 1970a & b; Barton-Browne, 1975), and a number of experiments involving lepidopterous larvae show clearly that such preferences can be induced and modified by prior experiences (Jermy et al., 1968; Feeny, 1970; Hanson, 1970, 1976; Hansen & Dethier, 1973). The degree to which plants and insects co-evolve has been intensively studied in several systems involving specific plant and lepidopteran groups (Ehrlich & Raven, 1964, 1967; Gilbert, 1971; Gilbert & Raven, 1975).

The polyphagous larvae of *Citheronia regalis* (Fabricius) (Saturniidae) (royal walnut moth) recently have been shown to exhibit differential growth and development on three genera of foodplants, representing three distinct plant families (Worth et al., 1979). These foodplants are persimmon (*Diospyros virginiana* L., Ebenaceae), sweetgum (*Liquidambar styraciflua* L., Hammamelidaceae), and wing-rib sumac (*Rhus copallina* L., Anacardiaceae). In that paper we postulated that persimmon and *C. regalis* were most compatible because of a long-standing, plant-herbivore relationship of neotropical origin; whereas, the latter two foodplants may have been exploited later by this moth species as its geographic distribution extended into temperate regions.

The present paper will explore this hypothesis further by examining differential growth and leaf utilization among first instar *C. regalis* larvae on these same three foodplants. The demonstration of such feeding preferences in the young larvae at the onset of their leaf feeding will strengthen this hypothesis by indicating that such preferences have become genetically incorporated in the moth strain we have been studying. The previous experiments had not been designed to test the early larval feeding responses of the royal walnut moth.

MATERIALS AND METHODS

Ten freshly eclosed *C. regalis* females from the Eldora (Cape May Co.), New Jersey stock were tethered overnight (Worth, 1980) to attract either wild, or marked and released, males during late June and July 1979. Thirty fertile eggs from each female were placed singly in petri dishes lined with moist filter paper. Ten eggs each were placed on small leaf cuttings of persimmon, sweetgum, and sumac. Thus, a total of 100 eggs (including ten from each one of the females) was tested on each foodplant (300 in all), thereby maximizing the genetic variance within each subclass of the experiment.

Nine of the P_1 females had been reared on persimmon, whereas the tenth had fed on sumac. Among the nine persimmon females, four bred with released males, which also had fed on persimmon, and the remaining five bred with wild males, whose larval foodplants were unknown. The tenth female (reared on sumac) likewise bred with a released male, which had been reared on persimmon. Due to the heavy predominance of persimmon among the P_1 moths of both sexes, we have treated the broods as a fixed, rather than as a random, variable in the statistical analyses employed in this paper.

The petri dishes were examined daily to determine whether or not the larvae were feeding and if they required additional leaf material. All larvae were observed until they either molted to the second instar, or until they died. Four larvae on each foodplant (a total of 12 in all) were chosen at random from among the 100 larvae, and their frass pellets were counted daily in an attempt to relate leaf intake and relative assimilation to the duration of the first instar. Due to time limitations, larger numbers of larvae were not used to obtain these data. The small size and weights of these hatchling larvae likewise kept us from obtaining accurate larval and fecal pellet weights. However, since the fecal pellets are of uniform size the numbers produced by each larva represent a good substitute criterion.

TABLE 1. Effects of foodplant and brood on the duration of first instar (days) among 262 *Citheronia regalis* larvae, with a 2-way ANOVA test and mean pair comparisons. The samples represent ten 1979 broods of the Eldora, NJ stock. (The sample sizes are given in brackets.)

		FOODPLANT		
	Persimmon (PR)		Sweetgum (SG)	Sumac (SM)
Duration of first instar ($\bar{x} \pm$ S.E.)	4.77 ± 0.10 [95]		5.04 ± 0.06 [100]	6.69 ± 0.18 [67]
ANOVA of duration of first instar (Foodplants and Broods are fixed)	df	Mean square	F-statistic	P
Between foodplants (F)	2	77.79	92.33	$P < 0.005$
Between broods (B)	9	3.58	4.25	$P < 0.005$
Interaction (F × B)	18	2.80	3.32	$P < 0.005$
Within subclasses (individuals)	232	0.84	—	—

If the means ($\bar{x}$) are ordered from largest to smallest, Duncan's Multiple Range Tests show that the three means differ significantly from each other: $PR < SG < SM$ ($P < 0.05$).

Our data have been analyzed using separate 2-way analysis of variance (ANOVA) tests on: 1) larval development time (duration of the first instar) and moth brood; and on 2) the number of frass pellets produced daily by the 12 larvae separated for this purpose. Due to an excessive number of zeros present in some of the daily frass count data, we performed a log transformation on the count data before analyzing them. Following both analyses, Duncan's Multiple Range Tests were used to test for statistical significance between individual mean pairs. Our statistical procedures follow those given by Freund et al. (1960).

RESULTS

Larval survival through the first instar was high (95–100%) on both persimmon and sweetgum but was much lower (only 67%) on sumac (Table 1). On the two aforementioned plants all larvae began to feed, as soon as their integuments had hardened. Those larvae placed on sumac, however, often wandered off the leaves, as if looking for another food source. Although a few began to feed immediately, the majority of larvae exhibited this wandering behavior. Many of the wanderers eventually returned to the sumac leaves and began to feed, although sparingly. Some never fed at all and starved to death while clinging to the presumably edible substrate. Each of the five larvae which died while feeding on persimmon was from a different brood. All fed well at first, and the cause of their deaths was not evident.

Table 1 shows the effects of foodplant and brood on the duration of first instar among the 262 surviving larvae. All three sample means

TABLE 2. Effects of foodplant and development time (days) on the number of frass pellets produced by 12 randomly chosen first instar *Citheronia regalis* larvae, with a 2-way ANOVA test and mean pair comparisons. The frass count data have been subjected to log transformation for analysis. (The total number of pellets is in brackets.)

		FOODPLANT		
		Persimmon (PR)	Sweetgum (SG)	Sumac (SM)
$\bar{x}$ No. of frass pellets produced over a six day period by four larvae on each foodplant	Actual $\bar{x}$ ± S.E.	18.17 ± 3.63 [436]	27.88 ± 5.00 [669]	30.25 ± 3.36 [726]
	$\bar{x}$ ± S.E. of transformed data	7.88 ± 1.01	12.22 ± 1.01	26.70 ± 1.01
ANOVA of No. of frass pellets produced (Foodplants and days are fixed)	df	Mean square	F-statistic	P
Between foodplants (F)	2	1.73	39.69	$P < 0.0005$
Between days (D)	5	3.16	72.54	$P < 0.0005$
Interaction (F × D)	10	0.85	19.61	$P < 0.0005$
Within subclasses (error)	54	0.04	—	—

Duncan's Multiple Range Tests show that the three $\log_{10}\bar{x}$'s (and consequently the other means, as well) differ significantly from each other: PR < SG < SM ($P < 0.05$).

are statistically significant from each other, with the larvae on persimmon exhibiting the most rapid growth and those on sumac the slowest growth rate. The between brood effects are also significant in this analysis, as is the foodplant × brood interaction term. Thus, larvae from separate broods exhibit distinctive growth rates, and these relative rates vary differentially, depending on the type of foodplant utilized.

The frass pellet count results are given in Table 2. The numbers of droppings produced daily during the first instar by four larvae feeding on persimmon is significantly lower than the number produced by the four larvae feeding on either sweetgum, or sumac. Once again, all three means exhibit statistical significance and, likewise, both the effect between days and the foodplant × days interaction term are statistically significant for the reasons given above.

The four larvae feeding on persimmon required only four days to complete the first instar, and they produced an average of 109 frass pellets. Those on sweetgum required five days in this stage and produced an average of 167 droppings. On sumac the first instar interval lasted six days, and the four larvae had a mean production of 182 fecal pellets.

DISCUSSION AND CONCLUSIONS

These results confirm and extend the conclusions of our earlier experiments. Persimmon represents the optimal foodplant for the Eldora, New Jersey stock of *C. regalis* when compared to either sweetgum or wing-rib sumac. That sweetgum occupies an intermediate position is more clearly shown than in our former experiments, while sumac stands out as an inferior food resource. The moth larvae exhibit differential responses to these three foodplants immediately as they begin to feed. Our results further support the hypothesis that the persimmon-*Citheronia* relationship is one of long standing and one which has become highly adaptive.

The frass counts suggest that the three foodplants provide different nutritional values, since larvae on persimmon did not have to eat as much to attain full size. Larvae on sweetgum had to eat half again as much as the former ones did to achieve the same growth. Although these tiny larvae were not weighed or measured, by inspection those on persimmon and sweetgum attained the same average size, while those surviving on sumac, despite feeding longer and more voluminously, were smaller when entering their first ecdysis. Such differential utilization and assimilation of the three foodplant genera by freshly hatched first instar larvae strongly suggests that the New Jersey strain of *C. regalis* is genetically more compatible with persimmon than it is with either of the two other foodplant genera.

These findings suggest that the food substances and nutrients required by this larval strain of *C. regalis* can be obtained more easily from persimmon than from either sweetgum or wing-rib sumac. The food compounds and nutrients ingested from leaves belonging to the later two plants are more difficult for the larvae to extract and utilize metabolically. Consequently, the *regalis* larvae are forced to expend more energy ingesting greater amounts of leaf material to accomplish the same amount of assimilation and growth when feeding on sweetgum or wing-rib sumac, thus, reducing their ecological efficiencies as plant predators.

Also, repellents such as toxic chemicals are present in sumac, despite the ability of some larvae to subsist on this plant. Perhaps it is significant that some of the other species of *Rhus* (*R. radicans* L., *R. toxicodendron* L., and *R. vernix* L.) possess recognized poisonous qualities (such as tannins and lacquer-like substances contained in the leaves). In spite of this, at least one neotropical species (*Citheronia splendens* (Druce) is known to feed on certain Anacardine genera such as *Shinus* and *Rhus* (Vasquez, 1944), but this insect also uses walnut (*Juglans*) and wild cotton (*Gossypium*) in Arizona (Ferguson, 1971).

The sumacs (*Rhus* spp.) represent a temperate zone genus of an extensive tropical Family, consisting of about 50 genera and nearly 400 species (Rodgers, 1920). There are 16–17 species of *Rhus* in North

America, nine of which are tree sized, including *R. copallina*. Both sweetgum and persimmon represent relict genera of trees, numerous species of which are found in the Eocene deposits of western North America, dating back about 55,000,000 years B.P. (Peattie, 1966). Today, the genera *Liquidambar* and *Rhus* represent mainly temperate plant groupings; whereas, *Diospyros* is more closely affiliated with tropical ebonies. This, we believe, is why persimmon represents the better foodplant for *C. regalis*, a temperate moth species having numerous neotropical relatives. We suspect that *C. regalis* has exploited the temperate plant genera more recently and that *Diospyros* represents the major foodplant of long-standing.

Larvae obtained from the moth that had been reared on sumac (and had mated with a male reared on persimmon) did not display a higher survival rate on sumac than offspring of moths in which both parents had been reared on persimmon. Whether better adaptation to sumac would take place over successive generations when both parents are reared on sumac is a question to which the senior author will next direct his curiosity.

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