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Effects of Larval Food-limitation on Vanessa cardui Linnaeus (Lepidoptera: Nymphalidae)

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ABSTRACT.—Larval food-limitation may be an important factor influencing growth of lepidopteran populations. We tested effects of food-limitation on larval growth of *Vanessa cardui* Linnaeus (Lepidoptera: Nymphalidae), the painted lady butterfly. *Vanessa cardui* is a major pest of corn and soybeans in Iowa, making it a species of special interest. Larvae of *V. cardui* were reared on a commercial diet apportioned to two food-limited and one control (*ad libitum*) treatment, and treatments were tested in three trials with varied temperature and onset of food limitation. Trial I was conducted at 18 C and food limitation started on day 12; Trials II and III were conducted at 22 C and food limitation started on days 7 and 12, respectively. Larvae were analyzed for potential differences in both larval and adult weight and time to pupation and emergence. In Trial I, there were more days to pupation and a prolonged adult emergence in the food-limited treatments. Control larvae developed and achieved maximal weights faster than food-limited larvae. Adult weights were also significantly higher in the control. Higher temperature in Trials II and III accelerated larval development. Early onset of food limitation at this higher temperature (Trial II) exacerbated differences among treatments, whereas later onset food limitation dampened these differences.

INTRODUCTION

Food-limitation may have a variety of consequences for lepidopteran populations, affecting demographic or genetic structure, and even causing local extinctions. In the case of a pest species, food-limitation could keep the population in check, whereas in the case of a rare species, food limitation could cause extinction. *Vanessa cardui* (the painted lady butterfly) is a serious pest of corn, alfalfa, sunflowers, beans and soybeans (Williams, 1970). This species is also known for large fluctuations in population size over time which can be caused by exhaustion of food supplies (Klots, 1951). The purpose of our study was to enhance the understanding of the role of food limitation and temperature on larval growth, adult weight and pupation and emergence times in *V. cardui*.

Previous studies have examined *Vanessa cardui* larval development under different temperature regimes (Poston *et al.,* 1977) and in a competitive setting (Poston *et al.,* 1978). However, food-limitation and temperature effects combined have not been examined. Poston *et al.* (1977) suggested that an optimal temperature for larval growth is approximately 24 C, the temperature at which maximal pupal weight and survival were achieved. Lower temperatures produced lower survival rates, and at temperatures below 15.5 C insects died before molting. In a later study, Poston *et al.* (1978) examined the effect of competition in conjunction with food-limitation. They found no significant effects of crowding on larval development, larval mortality or leaf consumption.

Most lepidopteran larval growth and survivorship studies in the past have focused on the genus *Pieris,* which is a major crop pest (*e.g.,* Ito *et al.,* 1960; Pimentel, 1961; Harcourt, 1966; Dempster, 1983). Studies on other genera have focused on larval growth as a function of host plant age and nutrient quality (Finke and Scribner, 1988). Studies on adult lepi-

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dopterans have focused on adult foraging behavior (Hainsworth and Hamill, 1993) or reproductive allocation as a function of adult diet (Hainsworth *et al.,* 1991; Boggs and Ross, 1993; Boggs, 1997).

We were primarily interested in determining what physiological flexibility *Vanessa cardui* larvae have for dealing with food-limitation. This included investigating both the effects of rearing temperature and time of onset of larval food limitation. We predicted that larval food-limitation would result in lower *adult* body mass and delayed emergence dates (from pupal to adult stages) and that lower temperatures would produce slower growing larvae. We did not choose to address the issue of fecundity because reproductive measures incorporate food-limitation at both the larval and the adult stages. When food-limitation occurs in the larval stage of an insect, body size can be affected immediately, whereas reproductive consequences will not be manifest until later life stages when the insect depends on other food resources.

METHODS

Gathering larvae from the field for the purpose of laboratory rearing can have considerable drawbacks. These may include presence of unknown diseases, suboptimal fertility, inability to locate larvae or limited abundance. *Vanessa cardui* are available commercially from laboratory supply companies; thus we used lab-reared *V. cardui* to minimize the effects of disease and maximize our sampling size. We avoided confounding results from competition or nutrient quality by rearing larvae in isolation and by using a complete diet medium. We measured the weight gain of individual *V. cardui* fed different amounts of a commercial food medium from Ward's Natural Biology (P.O. Box 92912, Rochester, NY, 14692). All individuals were provided *ad libitum* access to food until the third instar, when we began to measure specific amounts of food eaten by larvae assigned to the control treatment. We measured amounts remaining after feeding (wet weight) and calculated 60% and 80% of the amount eaten by control larvae, in grams of food per day. We fed these amounts to larvae assigned to the two food-limited treatments.

We conducted three trials testing larval food limitation. Trial I occurred in February 1996 and Trials II and III occurred in May 1996. Trial I began with 300 larvae spread over three treatments. Trials II and III were intended to occur as *one* set of 300 larvae, but had to be split into two trials because the larvae hatched on two different days. Thus, Trial II consisted of a set of 120 larvae, and Trial III consisted of 180 larvae. Each set was evenly divided over three treatments (60%, 80% and *ad libitum* food ration). Trial I began with 100 larvae in each treatment; Trials II and III included 40 and 60 larvae in each treatment, respectively. Trial I occurred under mean daily temperatures in the laboratory of 18 C (SE 0.3, from ten recordings of daily temperatures), whereas Trials II and III occurred under mean daily temperatures in the laboratory of 22 C (SE 0.4, from nine daily temperatures). These temperatures were suboptimal according to Poston *et. al.* (1977), but they were chosen so that we would have time to watch the effects of food limitation over time. Larvae were held in individual (100 ml) containers *en masse* in six large communal chambers. All food was separated into individual containers to avoid feeding interaction among larvae. Because the individual containers were small and covered except for a few small holes ($<$1 mm in diameter), we did not consider evaporative water loss of food to be a major issue, and thus used wet weight for food measurements.

We measured the larval weight gain by weighing larvae to the nearest 0.1 mg every three days in Trials I and II and daily in Trial III. We also noted the number of days to pupation, the number of days from hatching to adult emergence and the adult weight to the nearest milligram achieved for each individual. The food-limitation design was continued to the

stage at which larvae matured into the pupal stage. Adult weights were measured by subtracting the weight of a glassine envelope from the recorded weights of adult insects suspended in the envelope.

Statistical tests included one-way and two-way ANOVAs and repeated measures ANOVAs. The repeated measures ANOVA was used to test for effects of treatment and time on larval growth. However, we also ran separate one-way ANOVAs for particular dates so that we could use all of the observations for that date, not just the larvae that had complete data for all dates. One-way ANOVAs were conducted on larval weight at day 15 or 16 (depending upon the trial) because this was the time when treatments began to show differences, but it was before the time that larvae began to lose weight (before pupation) or sample size became reduced (due to pupation). In comparing results across trials, we used an Unweighted Analysis of Cell Means (Snedecor and Cochran, 1989) due to unequal sample sizes among trials.

RESULTS

Trial I.—The larval weight gains in Trial I showed a significant difference among treatments on day 16 (food-limitation began on day 12). On day 16, mean larval weight of the controls exceeded larval weight in the food-limited treatments. Mean larval weight also peaked sooner in the controls than in the 60% and 80% treatments (Fig. 1). A repeated measures ANOVA on days 7, 10, 13 and 16 showed significant effects of treatment, time and the interaction between treatment and time ($P < 0.001$ in each case). A one-way ANOVA testing for differences among the three treatments at day 16 was also significant ($F = 171.99$, $P < 0.001$, $df = 2$; no significant difference between the 60% and the 80% food-limited treatments). Larval weights peaked at approximately the same value, but it took 3 d longer for the food-limited treatments to reach maximum size. Adult emergence weights demonstrated a significant difference between the food-limited treatments and the control ($F = 19.06$, $P < 0.001$, $df = 2, 84$). Again, there was no significant difference between the 60% and the 80% food-limited treatments (Table 1). Similarly, the average time from hatching to adult emergence showed a significant effect of food-limitation: the control versus the 80% and 60% food-limited treatments were significantly different ($F = 122.23$, $P < 0.001$, $df = 2, 84$). See Table 1 for a summary of survival, pupation and emergence data.

Trials II and III.—Larvae for Trials II and III were reared in the same manner as for Trial I, but the mean temperature in the laboratory was 22 C (SE 0.4, from ten daily temperatures). Food limitation began on day 7 of Trial II and day 12 of Trial III. Using a repeated measures ANOVA on Trial II over days 7, 10 and 13, treatment was not significant, but time was significant ($P < 0.001$). The interaction between time and treatment was not significant. From days 7 through 10 there was no significant difference in the larval weight among treatments; differences became evident on day 13 (day 6 of food limitation) and these differences were statistically significant (one way ANOVA, $P < 0.01$). By day 16, the differences in larval weight were much more pronounced, and by the time larvae reached peak larval weight, each treatment was rank-ordered with controls having the greatest mass, followed by the 80% and then 60% food-limited treatments. However, statistical comparisons were not informative because the sample size had dropped dramatically with onset of pupation. Average adult weight was significantly different among treatments (ANOVA for all three treatments, $F = 11.05$, $P < 0.001$, $df = 2, 65$) but larval emergence dates were not.

Individuals from all treatments in Trial III attained comparable peak larval weights. Peak larval weight in the food-limited treatments, however, was reached later (1 and 2 d, respectively) in the 80% and 60% treatments (Fig. 1.). A repeated measures ANOVA on days 12,13,14 and 15 showed that time was again significant ($P < 0.001$), treatment was not, but

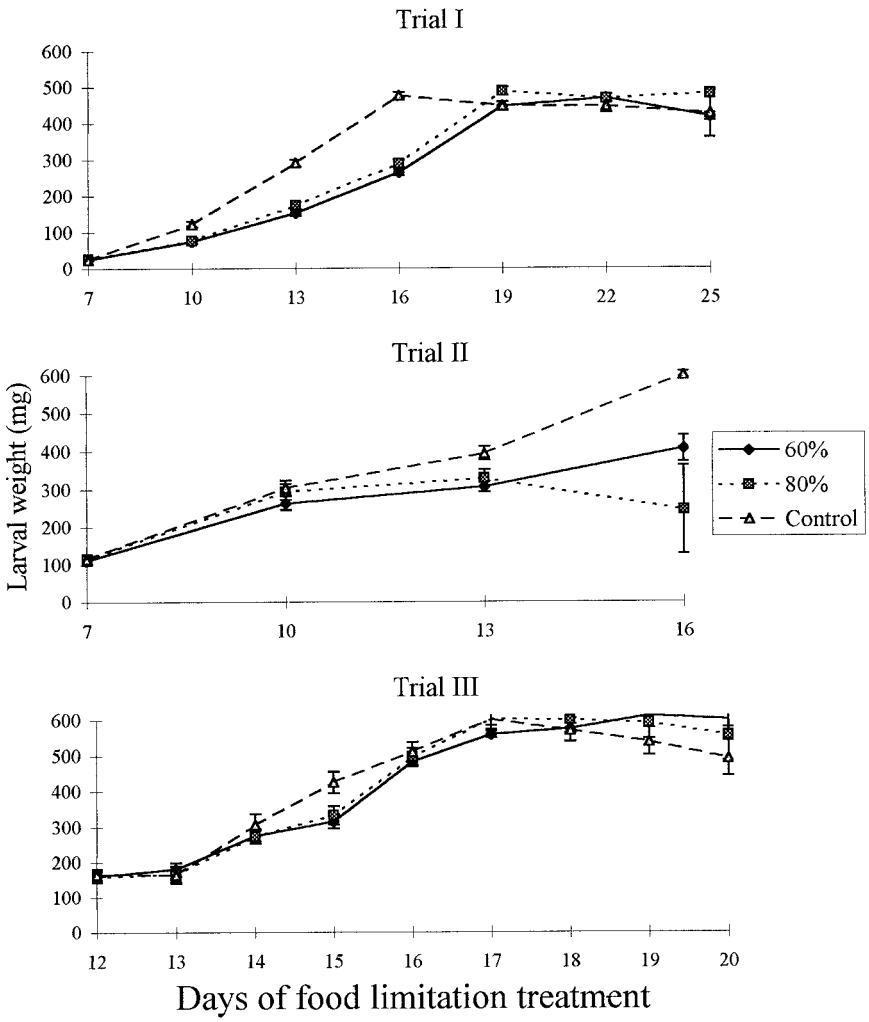

FIG. 1.—Summary of larval weights of *Vanessa cardui,* laboratory reared on 60% and 80% food-limited diets and *ad libitum* (control) diet fed to larvae in all 1996 laboratory trials. Diamonds represent 60%, squares represent 80% and triangles represent controls. Bars indicate SE

the interaction between time and treatment was significant ($P < 0.01$). Trial III showed results consistent with those of Trials I and II with respect to differences between control and experimental weights at day 15 (one way ANOVA comparing all three treatments, $F = 6.94$, $P = 0.001$, $df = 2, 149$). Controls were, on average, almost 100 mg heavier, with no significant difference between the 60% and 80% treatments. In Trial III, we also had the opportunity to compare the larval weight gain in control larvae with the amount of food consumed because we took daily weight measurements. Larvae increased in weight early in the experiment even though the greatest amount of food consumption occurred in the last

TABLE 1.—Pupation and emergence weight of all trials, with survival data

Trial: treatment	% Larval survival	Ave. days to pupation/SE	% Pupal survival	Ave. days to emergence/SE	Ave. adult wt. (mg)/SE
Trial I:					
60% (n = 100)	41.0 (n = 41)	25.6 0.2	30.0 (n = 30)	34.5 0.1	186.1 7.8
80% (n = 100)	38.0 (n = 38)	23.8 0.4	34.0 (n = 34)	33.9 0.3	199.2 8.5
Control (n = 100)	27.0 (n = 27)	19.8 0.3	21.0 (n = 21)	29.2 0.3	271.1 13.9
Trial II:					
60% (n = 40)	82.5 (n = 33)	15.7 0.4	70.0 (n = 28)	24.1 0.5	131.7 6.0
80% (n = 40)	77.5 (n = 31)	14.9 0.4	52.5 (n = 21)	23.1 0.5	135.8 4.8
Control (n = 40)	75.0 (n = 30)	14.4 0.2	45.0 (n = 18)	23.1 0.4	173.9 11.3
Trial III:					
60% (n = 60)	68.3 (n = 41)	17.5 0.3	45.0 (n = 27)	26.5 0.2	238.8 9.9
80% (n = 60)	55.0 (n = 33)	17.7 0.3	48.3 (n = 29)	26.5 0.2	268.8 9.3
Control (n = 60)	53.3 (n = 32)	17.9 0.4	16.7 (n = 10)	27.0 0.5	214.5 17.4

larval instar (Fig. 2). Despite the difference in pupal weights, the adult weights and times to emergence did not differ significantly from one another.

Our three trials consistently showed that control larvae gained weight faster than those in food-limited treatments. In two out of the three trials, adult weight was significantly higher in the controls. Temperature had significant effects on time to pupation; the mean pupation time was significantly longer in Trial I relative to Trial II or III (two-way ANOVA, trials $F = 18.59$, $P < 0.01$, $df = 2$). Survival rate was not significantly different among food-limited treatments, but it was different among trials, highest in Trial II (two-way ANOVA, trials $F = 8.61$, $P = 0.035$, $df = 2$). There was no significant difference in adult weight among trials, although Trial II had a tendency towards smaller adults.

DISCUSSION

As expected, there were more days to pupation and a prolonged adult emergence in Trial I of the food-limited treatments. However, this trend did not hold in Trials II and III. We noted, as in previous studies (Poston *et al.,* 1977; Poston *et al.,* 1978), that temperature greatly influenced the rate of larval development in *Vanessa cardui.* We now suspect that an interaction exists between food limitation and temperature stresses such that the effects of food-limitation are moderated by the temperature under which the experiment is conducted. The mean daily temperature in the laboratory was 4 C lower in Trial I than in Trials II and III. We assume this lower temperature was responsible for the unusually lengthy

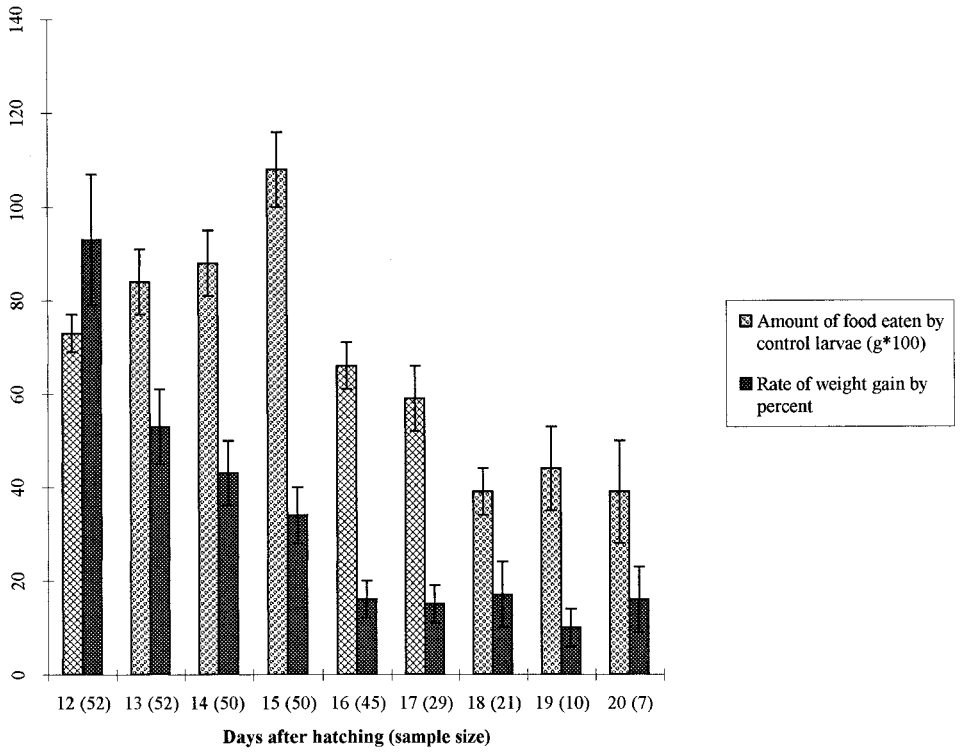

FIG. 2.—Trial III control larvae of *Vanessa cardui* reared *ad libitum*. The graph shows the relationship between the amount of food eaten (g *100 per day) in the light bars and weight gain (% increase from previous day's weight) in the dark bars. Bars around the mean indicate SE. Overall, mortality was low; sample size drops dramatically from day 16 to 17 due to pupation

emergence times we observed in Trial I. Poston *et al.* (1977) reported a mean of 23.5 days to pupation for larvae (food was not limited in that study) reared at 24 C. Our larvae took 14–25 d to reach pupation, depending upon the treatment. The date of onset of food limitation also had dramatic consequences. Trial III, which had a later onset of food limitation and a higher rearing temperature, produced larvae with the highest weights across treatments. Larvae at their earliest stages were more affected by food limitation than larvae at later instars. We did not expect this to be the case, given that one *V. cardui* larva eats 97% of the soybean leaf biomass it needs during the last two larval stages (Scott, 1986). Comparing the effects of time versus treatment, the repeated measures ANOVA showed that time was always a significant explanatory variable, but treatment was not. This is not entirely surprising, however, given that time should be significant if larvae are growing.

Vanessa cardui larvae have the flexibility to extend their growth period to achieve the necessary threshold weight before pupation. Food-limited larvae could not eat the same volume of food as controls on a daily basis, but by design of this experiment, they could continue eating longer until they pupated. In a field setting, food-limited larvae might have enough food to eat up to a certain point when the entire population exhausts the food supply. Larvae might not survive beyond that point. Evidently, larvae in the laboratory

merely changed their growth rate in response to food-limitation, but did not consistently exhibit lower peak larval mass, adult mass (except Trial I) or survival rates. Although slower larval growth is not necessarily a disadvantage in the laboratory, in a field setting food-limited larvae with an extended pupal stage would have longer exposure to predation. Larvae have poor escape abilities compared with adults, so their mortality would increase if food-limitation affected the larval rather than the adult stage. Comparing Trials II and III gives us some indication of the effects of timing of food-limitation, but we did not have any treatments that simply starved the larvae after a certain date.

Host plant quality is another consideration that would affect larvae in the field. *Vanessa cardui* is a larval host-plant generalist, feeding on dozens of host-plant species (Scott, 1986). The quality of the host plants can influence larval to pupal and adult survival of any insect species. Larvae that choose alternative host plants do not always derive sufficient nutrition and can accumulate adverse chemical compounds from the available, second-choice plants (Feeny *et al.,* 1985; Finke and Scriber, 1988). Finally, low nutritional content of host plants can cause larvae to modify behavior by eating more plant material, which increases exposure to predation (Fajer *et al.,* 1991).

An issue untested in this study is the effect of larval food-limitation on reproductive potential. The only indication that adults from food-limited larvae would lay fewer eggs was evidenced by the fact that in two of the three trials, adults weighed significantly less after being reared on food-limited diets as larvae. Previous research (Hainsworth *et al.,* 1991; Boggs and Ross, 1993; Boggs, 1997) has shown that adult diet affects fecundity.

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