Starvation Effects on Larval Development of Beet Armyworm (Lepidoptera: Noctuidae)[1]

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Abstract Sporadic starvation of herbivorous insect pests, such as the beet armyworm *Spodoptera exigua* (Hübner), often occurs due to host plant scarcity and dislodgement from host plants resulting from inclement weather, enemy attack, and other perturbations. We hypothesized that (1) starvation of beet armyworm in earlier instars would increase the possibility of undergoing supernumerary instars greater than would be the case for later instars; and (2) starvation of beet armyworm in earlier instars would prolong developmental time more than starvation in later instars. We tested these hypotheses by starving beet armyworm larvae for 48 h in the first, third, and fifth instars, and monitoring their development. Beet armyworms normally have 5 instars before pupation. Significantly more larvae underwent 6 larval instars (20.22 ± 4.35%) when starved for 2 d in the first instar compared with those starved for 2 d in the third (2.0 ± 2.00) and fifth instars (0.0), and relative to unstarved larvae (0.0). The number of instars that *S. exigua* larvae will experience can be predicted as early as the third instar-head capsule widths in this instar clearly differentiate for those destined to experience 5 and 6 instars. Developmental times of starved larvae also were prolonged compared with unstarved larvae. Developmental times (mean ± SE in days) of larvae starved in the first instar (20.32 ± 0.16) were significantly longer than those starved in the third (17.04 ± 0.20) or fifth instars (16.92 ± 0.10), or than unstarved larvae (14.62 ± 0.25). Timing of starvation significantly affected the pupal weight of larvae undergoing 5 instars. This suggests that (1) starvation of 2 d in very early instars (the first instar) increases the pupal weight at the cost of prolonged developmental time; and (2) starvation of 2 d in later instars (the fifth instar) leads to lower pupal biomass, presumably because of inability to compensate for the loss.

Key Words supernumerary development, developmental polymorphism, beet armyworm, *Spodoptera exigua*, starvation

Short-term starvation of herbivorous insect pests often occurs due to unavailability of host plants or dislodgement from the plant caused by inclement weather, enemy avoidance, or other perturbations (e.g., Briese 1986, Romeis et al. 1999, Naranjo and Ellsworth 2005, Asiimwe et al. 2006, Chen and Ruberson unpubl. data). Starvation may cause insect death but also may induce supernumerary instars and/or prolong the developmental time to compensate for short-term deficiencies. For example, *Manduca sexta* (L.) adds an additional molt if larvae are starved from the onset of the fifth instar for 3 d (Jones et al. 1980). Many insect species from different taxa have been observed to undergo supernumerary development due to starvation or suboptimal environmental conditions. Among them are: the gypsy moth [*Lymantria dispar* (L.)] (Leonard 1968, 1970), the wax moth [*Galleria mellonella* L.] (Krishnakumaran 1972,

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Pipa 1976, Boguś and Cymborowski 1984), the common cutworm [*Spodoptera litura* (F.)] (Yamanaka et al. 1975, Morita and Tojo 1985), the armyworm [*Leucania separata* (Walker)] (Iwao 1962), and the locusts *Locusta migratoria* L. and *Schistocerca gregaria* (Forskal) (Uvarov 1966).

Spodoptera exigua is a generalist insect pest with over 90 known host plant species (Pearson 1982). Early studies reported that the beet armyworm had 5 instars (Campbell and Duran 1929, Taylor 1931, Wilson 1934, Atkins 1960, Eveleens et al. 1973). However, Frost (1954), Fye and McAda (1972), and Pearson (1982) observed the occurrence of 5, 6, and even 7 instars. Pearson (1982) further recorded the proportion of *S. exigua* larvae that underwent different instars. He reported 45.7, 53.2, and 1.1% of the larvae reared with bean diet under constant temperature of 26.7°C and a photophase of 14 h had 5, 6, and 7 instars, respectively. Unlike gypsy moths where the number of instars is sex-linked (males have five, and females have six instars; Leonard 1970), the number of instars in *S. exigua* is not sex linked (Pearson 1982). The impact of starvation on the development of supernumerary instars in the beet armyworm is unknown. Small beet armyworm larvae are attacked by predators and often spin off the plants on a silken thread to avoid predation (Ruberson et al. 1994, pers. observ.). Beet armyworm larvae of later instars are highly mobile (Smits et al. 1987), and dislodgement from plants is often observed (pers. observ.). Thus, starvation may be a relatively common occurrence. Therefore, in this study, we investigated the impact of sporadic starvation at selected instars on supernumerary larval development of *S. exigua*. We hypothesized that (1) starvation of beet armyworm in earlier instars would increase the possibility of undergoing supernumerary instars greater than would be the case for later instars; and (2) starvation of beet armyworm in earlier instars would prolong the developmental time more than starvation in later instars.

Materials and Methods

Neonate *S. exigua* larvae less than 5 h old (collected from a laboratory colony in Tifton, GA, USA) were individually reared in 5-ml diet cups supplied with modified pinto bean diet (Burton 1969) in an environmental chamber at 25 ± 1°C and with a photoperiod of L14:D10. The beet armyworm colony had been maintained in the laboratory for 2 yrs (approx. 28 generations). Shortly after each molt (<16 h), beet armyworm larvae in selected instars (the first, third, and fifth instars) were deprived of diet for 48 h. The 48-h starvation time was selected because a preliminary trial indicated that a large number of the first-instar beet armyworm larvae died if starved longer than this interval, and it was considered to be sufficiently long to yield a measurable effect. Normal diet was restored thereafter, and they were monitored daily until pupation. Developmental instars were recorded daily, and cast head capsules were measured with a binocular microscope. A control, unstarved treatment (larvae fed throughout all instars) also was included. Each treatment (various developmental instars and control) was replicated 5 times, with 10 larvae per replicate. Thus, each treatment had a total of 50 larvae at the onset of the experiment.

All data were analyzed with ANOVA (PROC GLM in SAS) (SAS Institute 1999). Percentages of beet armyworm larvae undergoing 5 or 6 instars before pupation were arcsine (square-root) transformed before analysis. Developmental times and pupal weights were not transformed. Means were separated for significance using the least significant difference test (LSD) at $P < 0.05$ after the null hypothesis was rejected at $\alpha = 0.05$ level.

Results and Discussion

All *S. exigua* larvae in the study underwent 5 or 6 instars before pupation. The head capsule widths of *S. exigua* at different developmental instars are shown in Table 1 and are in agreement with those observed by Pearson (1982). The number of instars that *S. exigua* larvae will experience can be predicted as early as in the third instar, as the head capsule widths in this instar clearly differentiate for those destined for 5th and 6th instars (Table 1). The majority of *S. exigua* larvae had 5 instars before pupation, regardless of starvation or starvation timing, although 2-d starvation significantly affected the percentage of larvae experiencing 6 instars before pupation (F = 15.37; df = 3,16; $P < 0.0001$). Timing of starvation significantly affected the incidence of larvae undergoing 6 instars only when they were starved for 2 d in the first instar (Fig. 1). The mean (±SE) percentages of larvae experiencing 6 instars before pupation for larvae starved early the first, third, fifth instars and for the unstarved control larvae were 20.2 ± 4.35, 2.0 ± 2.00, 0.0 ± 0.00, and 2.0 ± 2.00, respectively. A starvation period of 2 d when beet armyworm larvae molted to the third and the fifth instar did not increase the number of larvae that went through 6 instars before pupation.

Starvation of beet armyworm larvae for 2 d also had a significant effect on developmental time from egg hatch to pupation (F = 164.35; df = 3,16; $P < 0.0001$) (Table 2), and developmental delays were affected by the instar in which starvation occurred. The developmental times of beet armyworm larvae starved early in the first instar were significantly longer than those starved early in the third and fifth instars and those that were unstarved. The developmental times of larvae starved early in third and fifth instars were also significantly longer than those of unstarved larvae, with the difference of approximately 2 d corresponding to the starvation period (Table 2). The difference in developmental times for larvae starved in early third and fifth instars was not significant.

Weights of pupae that underwent 5 and 6 instars before pupation were not different from each other when larvae were starved in the early first instars (F = 2.35; df = 1,36; P = 0.13; statistics of pupal weights on larvae starved in the early third and fifth instars, and control were not conducted because of limited individuals undergoing the sixth instar) (Table 3). However, timing of starvation significantly affected the pupal

Table 1. Head capsule widths of *S. exigua* larvae having 5 or 6 instars before pupation*

S. exigua category**	Head capsule width (mm)							
	2nd instar		3rd instar		4th instar		5th instar***	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Fifth	0.37	0.36-0.38	0.65	0.63-0.70	1.17	1.1-1.24	—	—
Sixth	0.37	0.36-0.39	0.56	0.53-0.59	0.87	0.83-0.90	1.30	1.26-1.33

* Head capsules of starved and control larvae combined.

** 5th and 6th denotes *S. exigua* larvae undergoing 5 and 6 instars before pupation, respectively.

*** Because cast head capsules of fifth instar *S. exigua* larvae were malformed measurements were not made.

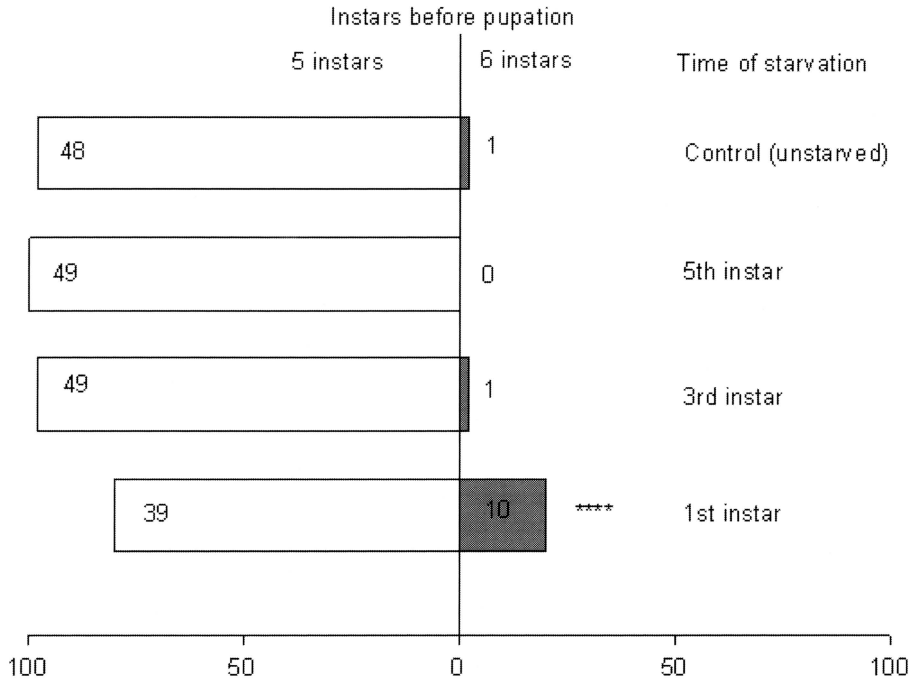

Fig. 1. Percentage of *S. exigua* larvae undergoing 5 and 6 instars before pupation following starvation of 48 h at the early stage (less than 16 h after molting) of different instars; numbers on or near the bar denote the number of larvae experiencing corresponding instars; ****$P < 0.0001$ among treatments (starvation timing).

Table 2. Developmental time (Mean ± SE) in days of *S. exigua* larvae from hatching to pupation

Timing of starvation	No. larvae	Developmental time (d)
1st	49	20.32 ± 0.16c
3rd	50	17.04 ± 0.20b
5th	49	16.92 ± 0.10b
Control*	49	14.62 ± 0.25a

* Unstarved; means followed by different lower-case letters were significantly different at $P < 0.05$.

weight for individuals experiencing 5 instars, with an inverse relationship between instar at time of starvation and pupal weight ($F = 7.61$; df = 3,91; $P < 0.0001$). This seems to indicate that (1) starvation of 2 days in very early instars (the first instar) increased the pupal weight at the cost of prolonged developmental time as shown in

Table 3. Pupal weights (Mean ± SE mg) of *S. exigua* larvae experiencing 2 d of starvation at various instars*

Timing of starvation	Pupal weight of individuals experiencing*	
	5 instars**	6 instars
1st instar	130.05 ± 2.10c	136.26 ± 4.00
N	27	10
3rd instar	118.30 ± 4.54ab	135.40
N	10	1
5th instar	113.41 ± 2.87a	—
N	24	
Unstarved	126.76 ± 2.60bc	146.00
N	34	1

* Not all pupae undergoing 5 instars were weighed; no larvae starved at the beginning of the 5th instar experienced a 6th instar;

** Mean weights of pupae undergoing 5 instars followed by different lower case letters were significantly different from each other at $P < 0.05$.

Table 2; and (2) starvation of 2 days in later instars (the fifth instar) led to lower pupal biomass, possibly due to more restricted time available for compensatory feeding and growth.

Unlike larvae fed on artificial diets, significantly more beet armyworm larvae in another study underwent 6 instars than 5 instars when fed on cotton plants receiving varying levels of nitrogen fertilization (all larvae underwent 5 or 6 instars before pupation; a mean of 96 ± 2.45 and 76 ± 5.10% for low and high N treatments underwent 6 instars, respectively), although significantly fewer larvae reared on high nitrogen plants experienced 6 instars (Chen et al. 2008, in press). This suggests that artificial diets used in the present study are of better nutritional quality than are cotton plants. Thus, starvation of larvae feeding on cotton plants may lead to more larvae undergoing at least one supernumerary instar than is the case on diet, even if starvation occurs in the early third or fifth instars.

Insect molting and metamorphosis are mainly controlled by nervous and endocrine systems. Starvation triggers the increase of juvenile hormone (JH) titer (Cymborowski et al. 1982). The presence of JH in the JH-sensitive period when the functional molting hormone 20-hydroxyecdysone is present inhibits the changes of the commitment of the epidermal cells, so that the same type of cuticle as the preceding one is produced (Klowden 2002, Heming 2003). However, the length and timing of starvation may be critical in triggering the supernumerary larval development because starvation of fifth-instar *M. sexta* larvae for 3 d led to higher frequency of larvae experiencing the sixth instar compared with unstarved larvae (Cymborowski et al. 1982).

Dislodgement of early-instar lepidopteran larvae due to inclement weather or enemy attack can be critically dangerous (Naranjo and Ellsworth 2005, Asiimwe et al. 2006) for various reasons, but prolonged starvation that can result from dislodgement

can lead to larval death, or can prolong the developmental time of surviving larvae to compensate for the developmental deficiency. Mortality of *S. exigua* in early developmental instars is generally high (Ruberson et al. 1994, Smits et al. 1987), but prolonged immature development may further increase *S. exigua* mortality due to biotic and abiotic factors as the slow-growth high-mortality hypothesis suggests (Feeny 1976). However, short-term starvation of later instars may have less significant ecological consequences than for younger larvae because late-instar *S. exigua* larvae are highly mobile and can recolonize host plants more readily, and because development was not as protracted in insects starved in the later instars as was the case for first instars. The significance of these findings for field populations of the beet armyworm are not presently known, but caterpillars are often found on the soil surface beneath plants in various cropping systems (Chen and Ruberson, pers. observ.), so it is possible that temporary starvation may be an important factor in shaping population dynamics of these species in the field.

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