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EFFECTS OF ASCORBIC ACID DEFICIENCIES ON LARVAE OF *LYMANTRIA DISPAR* (LEPIDOPTERA: LYMANTRIIDAE)

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ABSTRACT

We assessed the effects of ascorbic acid and total vitamin deficiencies on growth, food processing efficiencies and survival of larval gypsy moths. Artificial diet lacking ascorbic acid did not alter performance of fourth instars, whereas diet lacking a total vitamin mix marginally reduced growth. All vitamin deficient diets substantially reduced survival of fourth-fifth instars. Mortality occurred primarily during molting periods, providing further evidence of the putative role of ascorbic acid in cuticle formation.

That insects require dietary sources of water-soluble and fat-soluble vitamins is well-established, although the specific roles of such compounds, and their importance in insect nutritional ecology, are not. Such is particularly true for folivores of woody plants. Although the low nutrient content of tree foliage (relative to herbaceous foliage, Mattson and Scriber 1987) would suggest that vitamin deficiencies may be particularly important for tree-feeders, empirical studies with such insects are exceedingly few.

The role of ascorbic acid (vitamin C) in the nutritional ecology of the gypsy moth, *Lymantria dispar* L., is of interest for several reasons. First, ascorbic acid deficiencies have been implicated in predisposing gypsy moth larvae to one of their most important natural enemies, gypsy moth nuclear polyhedrosis virus (Lindroth et al. 1991). Second, ascorbic acid and other antioxidants may play important roles in the biological activation/deactivation of plant phenolics (Appel 1993, Felton and Duffey 1992), the dominant allelochemicals of preferred gypsy moth hosts. Third, procedures for mass-rearing of gypsy moths on artificial diets can be fully optimized only as the roles of particular dietary constituents are elucidated. At the time this study was conducted, ascorbic acid deficiency was under consideration as a contributor to Abnormal Performance Syndrome (APS, ODell 1990), which upon occasion interrupted rearing activities at USDA laboratories responsible for large-scale production of gypsy moth eggs and larvae.

Here we report the consequences of both ascorbic acid and total vitamin deficiencies on performance of larval gypsy moths. We addressed not only affects on growth and survival, but also proximate effects on food consumption and processing efficiencies.

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Table 1. Composition of test diets (% wet weight).

Component	HiVit+Asc	HiVit-Asc	+Asc	NoVit
Wheat germ	2.00	2.00	2.00	2.00
Casein (vitamin-free)	2.00	2.00	2.00	2.00
Cellulose (alphacel)	9.40	9.87	9.86	10.33
Mineral mix (Wesson's)	0.74	0.74	0.74	0.74
Sorbic acid	0.19	0.19	0.19	0.19
Vitamin mix[a]	0.93	0.46	–	–
Ascorbic acid	(0.47)[b]	–	0.47	–
Agar	1.39	1.39	1.39	1.39
Water (distilled)	83.36	83.36	83.36	83.36

[a] Hoffmann-LaRoche No. 26862 for HiVit+Asc: same minus ascorbic acid for HiVit-Asc.
[b] This value is included in the preceding column value for vitamin mix.

MATERIALS AND METHODS

We obtained gypsy moth egg masses from the Beneficial Insects Research Laboratory (USDA), Newark, Delaware. Larvae were reared in groups of 40–50 for the first three stadia; all rearing was conducted in a Percival® environmental chamber at 25°C with a 15:9 L:D photoperiod.

Artificial diets were modifications of the control diet described by Lindroth et al. (1991), which itself is a low wheat germ modification of the standard Bell diet (ODell 1985). We prepared four diets (Tables 1,2). The first contained the standard high concentrations of all vitamins, including ascorbic acid. The second was identical, with the exception that it contained no ascorbic acid. The third contained the standard amount of ascorbic acid but no additional vitamins. The fourth contained no supplemental vitamins. For ease of presentation these four diets will henceforth be referred to as HiVit+Asc, HiVit-Asc, +Asc, and NoVit, respectively. The HiVit+Asc and +Asc diets contained ascorbic acid at a concentration of 0.47% (fresh weight), a value at the upper end of the range of ascorbic acid concentrations in angiosperm foliage (mean of 0.16%, Jones and Hughes 1983). Diet mixtures were autoclaved to inhibit subsequent growth of mold; vitamins were added after diets were cooled to below 70°C. All insects were reared on the HiVit+Asc diet for stadia 1–3.

We performed two types of bioassays to assess the effects of ascorbic acid

Table 2. Composition of vitamin mix formulation.

Component	%
Vitamin A	3.39
Vitamin E	0.80
Vitamin B12	0.04
Vitamin B2	0.05
d-Pantothenic acid	0.10
Choline chloride	10.02
Folic Acid	0.02
Ascorbic Acid	50.10
Thiamin	0.02
Pyridoxine	0.02
Biotin	0.02
Niacin	0.10
Inositol	2.00
Dextrose	33.09

Table 3. Dietary effects on nutritional indices of fourth stadium gypsy moths (mean ± 1 S.E.)**

Diet	Duration (days)	RGR (mg/mg/day)	RCR (mg/mg/day)
HiVit+Asc	5.7±0.2^{a}	0.21±0.01^{a}	1.81±0.05^{a}
HiVit-Asc	5.5±0.3^{a}	0.20±0.00^{ab}	1.77±0.10^{a}
+Asc	5.0±0.2^{a}	0.20±0.00^{ab}	1.93±0.05^{a}
NoVit	5.5±0.2^{a}	0.18±0.01^{b}	1.99±0.08^{a}
P-value	0.197	0.029	0.105

Diet	AD (%)	ECD (%)	ECI (%)
HiVit+Asc	21.1±0.9^{a}	56.4±4.3^{a}	11.5±0.3^{a}
HiVit-Asc	19.5±2.5^{a}	63.6±9.0^{a}	11.1±0.6^{a}
+Asc	17.6±1.2^{a}	61.4±4.0^{a}	10.4±0.2^{a}
NoVit	13.4±0.6^{b}	70.2±4.0^{a}	9.3±0.3^{b}
P-value	0.005	0.357	0.001

**Within a column, means with different superscripts are significantly different ($P<0.05$). RGR = relative growth rate, RCR = relative consumption rate, AD = approximate digestibility, ECD = efficiency of conversion of digested food, ECI = efficiency of conversion of ingested food.

and general vitamin deficiencies on gypsy moth performance. Feeding trials with fourth instars were conducted to determine dietary effects on growth and consumption rates and food processing efficiencies. Newly molted fourth instars (80–100 mg) were placed individually into 28 ml plastic cups containing a cube of one of the four test diets. We assayed twelve insects (replicates) per diet. Food was replaced at 2–3 day intervals, or more frequently if needed, until completion of the fourth stadium. Newly molted fifth instars were frozen, then larvae, frass and remaining food were dried (65°C) and weighed. Initial dry weights of larvae and food were estimated using proportional dry weights derived from subsets of larvae and food not used in the experiment. We calculated nutritional indices based on standard formulas (Waldbauer 1968, Scriber 1977). Relative rates of growth and consumption were calculated based on initial rather than mean insect weight (Farrar et al. 1989).

Our second bioassay assessed the effects of vitamin deficiencies on insect survival, development, and pupal weights. Newly molted fourth instar larvae (12–16 per replicate, six replicates per diet) were placed into 600 ml plastic rearing containers and fed one of the four test diets. We recorded survival rates and pupal weights (3–4 days post pupation) until all larvae had either died or pupated.

Results from both bioassays were analyzed by one-way analysis of variance (ANOVA) using SAS statistical software. Treatment means were compared by the Student-Newman-Keuls multiple range test (SAS Institute 1985).

RESULTS

Performance of fourth instar gypsy moths was largely unaffected by dietary vitamin treatment (Table 3). Development rates (stadium duration) and consumption rates did not differ among treatments. Growth rates of larvae fed the NoVit diet were 14% lower than those of larvae fed the HiVit+Asc

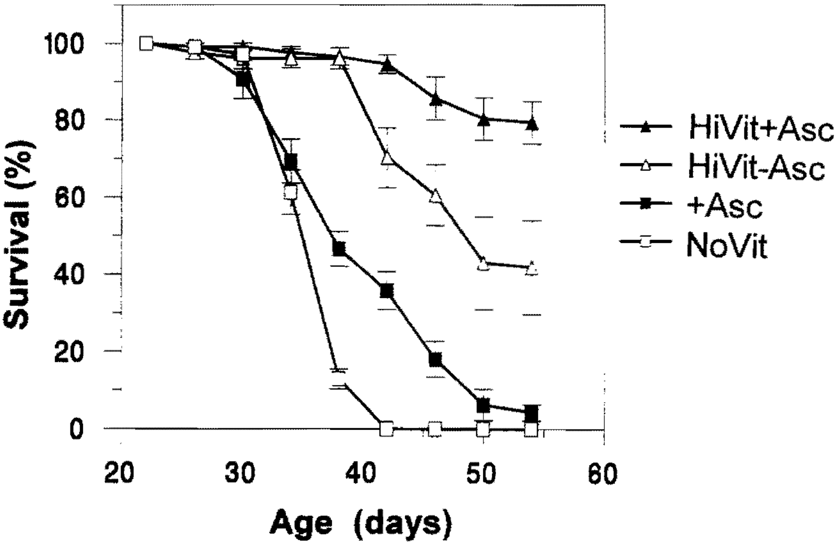

Figure 1. Survival of fourth and fifth instar gypsy moths on control and vitamin deficient diets. Vertical lines indicate ± 1 S.E.

diet, but not significantly lower than those of larvae on other vitamin deficient diets. Larvae on the NoVit diet also exhibited reduced food processing efficiency; approximate digestibility decreased 36% relative to that of insects on the HiVit+Asc diet.

In contrast to results from the fourth instar feeding studies, we found striking effects of vitamin deficiencies on mortality of fourth/fifth instars (Fig. 1). Elimination of ascorbic acid from a high vitamin diet reduced survival rate from 79 to 42%. Only 4% of larvae fed the +Asc diet survived through pupation, whereas none of the larvae on the NoVit diet survived through pupation. Moreover, mortality occurred earlier for insects on the NoVit diet than for those on the +Asc diet, and earlier for larvae fed the +Asc diet than for those fed the HiVit-Asc diet. Many of the insects reared on the NoVit diet died in the molt to the fifth stadium. Most mortality for insects fed the HiVit-Asc diet occurred during the metamorphic molt (larva to pupa). Interestingly, development times and pupal weights were not negatively affected for the fraction of insects fed the HiVit-Asc diet that successfully pupated (Table 4).

DISCUSSION

In general, vitamin deficient diets did not markedly alter growth and developmental rates of gypsy moth larvae. The single exception was the NoVit diet, but even those effects were marginal. In an earlier study with the HiVit+Asc and NoVit diets, the latter caused a 27% reduction in growth due to a decrease in the efficiency of conversion of digested food (ECD) (Lindroth et al. 1991). Growth of other lepidopteran species appears to be more sensitive to ascorbic acid deficiency than what was exhibited by gypsy moth larvae.

Table 4. Dietary effects on development times and pupal weights of gypsy moths (mean ± 1 S.E.)§†

	Duration (days)		Weight (mg)	
Diet	Males	Females	Males	Females
HiVit+Asc	37.3 ± 0.2^a	42.5 ± 0.4^a	541 ± 10^a	1559 ± 47^a
HiVit-Asc	38.9 ± 0.3^a	42.9 ± 0.3^a	550 ± 12^a	1298 ± 35^a
P-Value	0.064	0.863	0.827	0.192

§Within a column, means bearing different superscripts are significantly different ($P < 0.05$).
†Poor survival of larvae on the +Asc and NoVit diets precluded incorporation into this table.

Shao et al. (1993) reported greatly reduced growth and 0% survival (to pupation) of *Manduca sexta* Joh. larvae reared on artificial diets lacking ascorbic acid. Navon et al. (1985) found that within 72 hours following ascorbic acid deprivation, consumption rates increased and growth rates decreased in *M. sexta* and *Spodoptera littoralis* (Boisduval).

The most pronounced effect of ascorbic acid (and total vitamin) deficiency in our study was on larval mortality. This result was also observed by Lindroth et al. (1991), who found that vitamin-deficient larvae succumbed to NPV infection. Such was not the case in this study, as microscopic examination revealed no polyhedral inclusion bodies.

Mortality occurred primarily during periods of molting. Elevated mortality during the molt is consistent with results of other studies with lepidoptera (Navon 1978, Kramer and Seib 1982). This phenomenon has been attributed to the putative role of ascorbic acid in cuticle formation, particularly in collagenesis and control of diphenoloxidase activity (Navon 1978, Navon et al. 1985). Rapid onset of ascorbic acid deficiency symptoms in actively feeding *M. sexta* and *S. littoralis* larvae, however, led Navon et al. (1985) to investigate other mechanisms of action. They concluded that debility may be linked to disruption of ion and water transport processes.

That ascorbic acid plays multiple roles in insect biochemistry/physiology is becoming increasingly clear. The sensitivity of different biochemical/physiological processes to ascorbic acid deficiency, however, appears to differ among insect species. Unlike the results of Navon et al. (1985) for *M. sexta* and *S. littoralis*, only molting processes appear to be highly susceptible to ascorbic acid deficiency in gypsy moth larvae.

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