GROWTH, FOOD CONSUMPTION, AND NITROGEN AND LIPID COMPOSITIONS OF THE COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA*, (COLEOPTERA: CHRYSOMELIDAE), AS A FUNCTION OF THE NITROGEN SUPPLY OF ITS HOST PLANT

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ABSTRACT

Experiments were designed to investigate effects of nitrogen (N) fertilizer supplied to potato plants on growth and food utilization of larval Colorado potato beetles (CPB). Dry mass gain, dry mass consumption, and efficiency of conversion of ingested food (ECI = dry mass gain/dry mass consumption) were determined for the entire larval stage and nitrogen and lipid compositions for larvae just prior to pupation. Dry mass gain and N composition were measured in both laboratory and greenhouse reared larvae, dry mass consumption and ECI in laboratory reared larvae, and lipid composition in greenhouse reared larvae.

Significant linear increases with N supply were found for dry mass gain of greenhouse reared larvae, but not laboratory reared larvae, and for N composition of laboratory reared larvae, but not greenhouse reared larvae. No significant effects were demonstrable for consumption and ECI of laboratory reared larvae, or lipid composition of greenhouse reared larvae. These results suggest that N supply can affect performance of CPB larvae but that the effects vary with small differences in rearing conditions.

Key Words: Colorado potato beetle, ECI, food consumption, growth, *Leptinotarsa decemlineata*, lipids, nitrogen, potato.

J. Entomol. Sci. 24(1): 62-69 (January 1989)

INTRODUCTION

Nitrogen is, of course, an essential component of the tissues of all organisms and is known to limit individual and/or population growth in many terrestrial animals (Mattson 1980; Scriber 1984). Herbivores, including insects, are especially likely to be N limited since plant tissue is generally much lower in N than animal tissue (Scriber 1984). Numerous studies have shown growth, reproduction, and survival of phytophagous insects to be positively correlated with the N content of their food (reviewed by McNeill and Southwood 1978; Mattson 1980; Scriber 1984; Slansky and Scriber 1985). Additional work, reviewed by Tingey and Singh (1980), has documented the positive effects of soil N concentration on insect fitness, presumably through its intermediate effect on plant N content, although a variety of plant physiological responses to N fertilization could influence insect feeding and survival (e.g., Shaw and Little 1972). On the other hand, N is also a component of a variety of plant antifeedants and toxins and may be present as non-utilizable nitrates (Jansson and Smilowitz 1986a). Thus it is not surprising that some studies have shown no correlation or a negative correlation between insect performance and food N content (Scriber 1984) or soil N (Tingey and Singh 1980).

Previous work on the CPB amply illustrates the complexity of effects of N and N - containing compounds on insect herbivores. Hare (1983) reported that suitability of nightshade, *Solanum dulcamara*, as a host for the CPB varies seasonally and that larval survival is positively correlated with extractable protein in nightshade foliage during late spring to mid - summer but negatively correlated with glycoalkaloid content during late summer. Jansson and Smilowitz (1985) showed that variation in the level of N fertilizer applied to potato, *Solanum tuberosum* var. Katahdin, had no significant effect on abundance of CPB under field conditions, while a slight but significant negative correlation existed between beetle development rate and foliar N content. In contrast, the same authors (Jansson and Smilowitz 1986b) found that when different potato cultivars were compared, CPB abundance was positively correlated with foliar N content.

In the work reported here we investigated the effects of varying levels of N fertilizer applied to greenhouse - cultivated potato on growth, food consumption, and N and lipid compositions of CPB larvae reared under partly controlled conditions either in the laboratory or greenhouse. Our findings indicate that N supplied to food plants may have variable effects on CPB.

METHODS AND MATERIALS

I. Potato Cultivation

Individual seed pieces of the cultivar 'Superior' were planted approximately 1" below the surface in 7"D × 7.75"H pots containing equal volumes of sand. The pH of the sand was low enough to severely inhibit plant growth so the sand in each pot was washed with 1 liter of calcium carbonate solution (0.083 g/l); the treatment allowed plants to sprout and grow normally at an unmeasured pH. Nutrients were not added until all plants had emerged. Thereafter, until two weeks prior to beginning an experiment, all plants were treated (fed) every third day with 0.65 l each of Hoagland solution (R. Flannery, pers. comm. 1981) diluted to 34.4% of the standard formula, but ammonium nitrate was added to elevate the nitrogen level to 150 ppm to promote rapid plant growth during this pre -experimental period. For two weeks immediately prior to and during the course of an experiment, nutrients were supplied with 0.65 l of 34.4% Hoagland solution in which N was adjusted to 75, 150, 225, or 300 ppm by addition of ammonium nitrate. Treatments were allocated to plants in a Latin square experimental design.

Plants fed to laboratory reared larvae were grown under natural illumination supplemented from 0600 to 2200 h every day by two lamps with Duro - Test Vita Lite® flourescent tubes, 36" above the bench where the plants were growing. Since experiments were run sequentially from 1/81 - 8/81, the quality of illumination changed seasonally. Foliage for greenhouse reared larvae were grown without artificial lighting. Plants were watered with equal volumes of tap - water as needed. Aphids and whiteflies were controlled with pirimicarb (wettable powder, 0.3 g/l) and resmethrin (24.3% emulsifiable concentrate, 2.4 ml/l), respectively; plants were never treated with insecticides later than one week prior to the start of an experiment. Temperature ranged from 20 - 35°C.

II. Larval Rearing

A. Laboratory Experiments

For detailed studies of larval growth and food consumption larvae were kept in the laboratory on potato foliage in one pint (0.473 l) mason jars closed with the usual metal ring but with the metal lid replaced by a 9 - cm circle of filter paper.

To insure that at least 1 survived to pupation, 3 or 4 first - instar larvae (less than one day old) were placed in each jar. Randomly selected individuals were removed as they developed, so each experiment ended with one larva per jar. Each jar was supplied with a potato leaf in an Aqua - Pic® to maintain turgor. The jars were placed on one shelf of a Hythermco® temperature cabinet with treatments assigned to jars in a Latin square experimental design and maintained at 24.5 ± 1.5°C and 16L : 8D photoregime.

For the first experimental replication newly eclosed larvae from one egg mass were randomly assigned to all treatments within a block. For the second and third replications larvae from four egg masses were separated into four weight classes. Randomly selected larvae from each weight class were assigned to treatments and blocks so that each treatment within each block had a larva from each weight class.

B. Greenhouse Experiments

To obtain numbers sufficient for nitrogen and lipid analyses, larvae were also reared in the greenhouse. Plants were treated with N at 75 - 300 ppm in 75 ppm increments. Each group of plants comprising a treatment level was isolated within blocks to prevent larvae from wandering among plants of different treatments. Completely randomized blocks were run serially in time, each occupying the same greenhouse area.

Newly eclosed larvae from entire egg masses were assigned to a plant and transferred to fresh plants within the same treatment as necessary. Fourth instar larvae were removed and placed in open, cylindrical, clear - plastic, containers (up to 20 larvae per container) with paper towels to absorb moisture, and three or four Aqua - Pics®, each with a potato leaf from an appropriately treated plant. Each container with final instar larvae was adjacent to the group of plants from which the larvae had been removed. Larvae were left in containers until they appeared ready to pupate (i.e., large, bright orange, and not staying on foliage), then killed and stored at - 18°C in capped vials.

III. Estimation of Dry Mass Gain of Larvae

All weighing was done with a Mettler® balance, accurate to 0.05 mg, and all drying with a Precision Scientific® oven at 63 ± 3°C for 18 hours, except as noted. The order of weighing was randomized in a Latin square experimental design.

In a preliminary experiment, 175 newly hatched larvae were weighed together before and after drying at 82 ± 3°C for 18 hours. The mean percent dry mass of larvae (26.8) was used to estimate dry mass of newly eclosed first - instar larvae during subsequent experiments.

After cessation of feeding and clearing of the gut, experimental larvae were killed and stored in capped vials at - 18°C until they were dried and weighed. Dry mass gain of laboratory reared larvae was calculated as the difference between the estimated initial dry mass and the final dry mass. We did not estimate initial dry mass of greenhouse reared larvae, as the initial mass (about 0.1 mg) is negligible compared to the prepupal mass.

IV. Estimation of Dry Mass Consumption of Potato Leaf Foliage by Laboratory Reared Larvae

Preliminary experiments established that the percent dry matter of the terminal leaflet of potato leaves was highly correlated with dry matter of the remaining leaflets (Spearman rank correlation coefficient, $r_s = 0.886$, $p << 0.0005$). Use of the terminal leaflet consistently underestimated the percent dry matter of the remaining leaflets by 3 - 5%.

To estimate dry mass consumption of potato foliage, the first fully expanded leaf below the growing tip of a stem, one per plant, was excised. Each was inserted individually into an Aqua - Pic® and stored in a temperature cabinet overnight at 15°C to allow wilted leaves to regain complete turgor. Thereafter, terminal leaflets were excised and weighed, then the remainder of each leaf was promptly weighed and returned to the Aqua - Pic . The newly weighed leaves were exchanged with the older leaves in the mason jars and the larvae transferred. Old foliage was left in the Aqua - Pics for about four hours, then cleaned of feces by rubbing and brushing. The remaining leaflets and the petiole were immediately weighed separately and the excised leaflets subsequently dried and reweighed. Dry mass consumption of each leaf was estimated using the formula:
[(fresh mass without apical leaflet - fresh mass of petiole) × (% dry matter of apical leaflet/100)] - dry mass of the uneaten leaflets.

V. Determination of Nitrogen Compostion of Larvae

Larvae were removed from cold storage, dried, and stored in capped vials. For the first N - determination replication, laboratory - reared larvae from the experiments previously described were combined into four groups, each from plants treated with the same level of N. For the second N - determination replication, greenhouse reared larvae from the two experimental blocks were appropriately grouped. In each case, random samples of three larvae, each weighing about 0.1g, were drawn from each group. These samples were analyzed for total N using microKjeldahl procedures, (D. Markus, pers. comm. 1981). Two aliquots were taken from each sample and the levels of N determined with a Technicon® autoanalyzer.

VI. Determination of Lipid Composition of Larvae

Samples of 5 - 7 greenhouse reared larvae, previously dried and stored in capped vials over Drierite®, were weighed, then homogenized in a Ten Broeck® 40 ml hand tissure grinder, to which was added 25 ml of chloroform/methanol (C/M) mixture (2:1, v/v). Lipids were extracted according to the method of Folch et al. (1957) and dried according to Bligh and Dyer (1959).

RESULTS

The data are summarized in Tables 1 and 2. Treatment effects were tested by ANOVA, based on Latin squares experimental designs except for dry mass gain and ECI of laboratory reared larvae; the latter are based on a completely randomized design because the existence of significant treatment by block interactions in these analyses violated the assumptions for tests of significance under the original Latin squares allocations.

Significant differences attributable to treatment (N) effects were found for N composition of laboratory reared larvae (Table 1) and for dry mass gain of greenhouse reared larvae (Table 2); both increased with increasing levels of N applied to plants, and in both cases data fit linear regression models, implying significant differences among all means in each case (Chew 1976). No significant N effects were found for dry mass gain, dry mass consumption, or ECI of laboratory reared larvae (although there were marked negative trends among the means for dry mass gain and consumption and a positive trend for ECI; Table 1), or for N or lipid composition of greenhouse reared larvae (Table 2). We point out that the magnitude of the change in mean dry mass gain in laboratory reared larvae was greater than that in greenhouse reared larvae, although of opposite sign; since the variance associated with the means for each treament was roughly the same in

both groups, we do not dismiss the possibility that the failure to obtain significant results in the laboratory reared larvae is a consequence only of the much smaller sample sizes in that experiment.

Table 1. *Nitrogen treatment effects on laboratory reared larvae: data summary and statistical analysis.*

Measured Quantity	Nitrogen Level (PPM)				F	P
	75	150	225	300		
Dry Mass Gain (mg)						
$\bar{X}$	38.64	37.44	36.62	35.91	0.54	> 0.25
95% C.I.	35.18-42.10	33.98-40.90	33.16-40.08	32.45-39.37		
Range	31.69-50.11	30.07-44.19	25.69-43.97	30.32-44.60		
Variance	37.01	19.78	21.86	13.00		
N	13	13	13	13		
Dry Mass Consumption (mg)						
$\bar{X}$	170.95	162.27	151.97	151.88	1.08	> 0.25
95% C.L.	151.77-190.13	143.09-181.45	132.79-171.15	132.70-171.06		
Range	124.22-202.91	125.93-200.15	113.29-201.29	123.10-209.00		
Variance	494.77	515.59	574.76	577.67		
N	13	13	13	13		
E. C. I. (%)						
$\bar{X}$	27.66	27.75	31.24	30.61	1.83	> 0.25
95% C.I.	23.67-31.84	23.76-31.94	27.08-35.55	26.48-34.90		
Range	16.1 -37.1	22.3 -33.5	21.0 -39.0	18.1 -37.7		
Variance	5.21	1.18	2.28	3.89		
N	13	13	13	13		
N - Composition of Larvae (%)						
$\bar{X}$	8.79	9.33	9.25	9.57	4.44	< 0.05
95% C.I.	8.28-9.31	8.80-9.86	8.73-9.79	8.98-10.18		
Range	8.4 -9.3	8.9 -9.5	9.0 -9.5	9.3 - 9.9		
Variance	6.49×10^{-6}	1.25×10^{-6}	6.73×10^{-7}	1.67×10^{-6}		
N	5	5	5	4		

Significant differences also were attributable to factors other than N. ANOVA of the ECI data showed a significant replications effect. The untransformed grand means of the three experimental replications were 27.3, 29.5, and 31.7% (not corrected for errors in estimating the dry mass of fresh leaflets). A significant block effect in the ANOVA of the lipid composition of greenhouse reared larvae data indicates that the transformed grand means of each block, 15.5 and 16.2%, are significantly different. The transformed grand means of the two experiments investigating N composition of larvae were significantly different (Student's $t = 10.26$, $p << 0.0005$); the untransformed grand mean of laboratory reared larvae was 9.25%, that of greenhouse reared larvae was 10.94%.

DISCUSSION

Latheef and Harcourt (1972) previously reported dry mass gain and ECI for CPB larvae reared on potato and tomato. Our larvae reached slightly higher final masses than theirs on potato (roughly 37 vs 32 mg) and appreciably higher masses

than their tomato - reared larvae (about 19 mg). Their ECI values on potato (about 45% for the entire larval period) were higher than ours, although their tomato reared larvae showed much reduced ECI (about 15%). The experimental technique used by these authors differed from ours, as did the potato variety; they did not indicate fertilizer levels applied. Apparently our data on N and lipid compositions are the first to appear for CPB larvae.

Table 2. *Nitrogen treatment effects on greenhouse reared larvae: data summary and statistical analysis.*

Measured Quantity	Nitrogen Level (PPM)				F	P
	75	150	225	300		
Dry Mass Gain (mg)						
$\bar{X}$	36.98	37.75	38.33	38.44	4.15	< 0.05
95% C.I.	35.89 - 38.07	36.77 - 38.73	37.30 - 39.36	37.35 - 39.53		
Range	22.22 - 48.92	25.09 - 49.06	26.93 - 51.33	23.92 - 49.43		
Variance	19.67	28.84	24.99	20.49		
N	79	97	89	79		
N - Composition of Larvae (%)						
$\bar{X}$	10.93	10.90	10.68	11.24	0.78	> 0.25
95% C.I.	10.30 - 11.58	10.27 - 11.55	10.05 - 11.32	10.60 - 11.90		
Range	9.9 - 11.8	9.7 - 12.2	10.1 - 11.4	10.3 - 12.3		
Variance	4.26×10^{-5}	1.44×10^{-4}	8.39×10^{-6}	5.18×10^{-5}		
N	6	6	6	6		
Lipid Composition of Larvae (%)						
$\bar{X}$	15.80	15.96	15.66	16.08	0.20	> 0.25
95% C.I.	15.21 - 16.41	15.40 - 16.52	15.06 - 16.26	15.53 - 16.64		
Range	15.1 - 17.2	14.3 - 17.4	14.8 - 16.7	14.4 - 17.4		
Variance	3.51×10^{-5}	1.08×10^{-4}	9.95×10^{-6}	8.65×10^{-5}		
N	8	9	8	9		

We cannot suggest a specific reason for the apparently disparate effects of N on growth in our two experiments. Changes in plant composition with changes in season or more subtle, uncontrolled changes in growing conditions, or as a result of excised foliage being used primarily in the laboratory experiment, may underlie the different results. Our principal conclusion is that N fertilization levels experienced by host plants can influence growth of CPB larvae but the nature of the effects is dependent on other unknown factors.

The changes in N composition of the laboratory reared larvae show that, even in the absence of increased mass gain, increased N supplied to host plants can enhance the ability of CPB larvae to accumulate N; this might tend to increase fitness by increasing fecundity, for example, independently of changes in mass (Mattson 1980). Again we are unable to pinpoint reasons for the contrasting failure to find a significant relationship between N composition and fertilization rate in greenhouse reared larvae. Since the N content of the greenhouse reared larvae was higher at all treatment levels than that of laboratory reared larvae, it seems plausible that in the greenhouse experiment the larvae could attain optimum tissue N composition at all levels, while this parameter remained suboptimum in the laboratory experiments. The trends toward reduced consumption and increased ECI with increasing N fertilization in laboratory reared larvae could reflect an

attempt by the CPB larvae to stabilize N accumulation, as may occur in other phytophagous insects (Mattson 1980; Slansky and Feeny 1977; Slansky and Scriber 1985). Clearly, however, our data need additional confirmation.

In summary, we conclude that N supply to and presumably N content of host plants can affect performance of CPB larvae but that the relationship is sensitive to a variety of other variables. Our results are in general agreement with the results of Jansson and Smilowitz (1985, 1986b) where several measures of population performance of CPB on a single potato cultivar (Katahdin) were either unaffected by or negatively correlated with level of N fertilizer applied to the plants; however, CPB abundance on different cultivars was positively correlated with foliar N level. They suggested that differences in N dynamics within and between cultivars could account for the differences in the beetles' responses. Likewise, Hare (1983) found seasonally variable responses to levels of several N-containing compounds in *Solanum dulcamara*, probably as a result of a complex interaction of effects of these phytochemicals.

ACKNOWLEDGMENTS

Our sincere thanks to Dr. J. Lashomb for help at many stages of this research, including critical reading of the manuscript. We also thank Drs. S. Ahmad, W. Carey, R. Flannery, and D. Markus for assistance with various experimental techniques. New Jersey Agricultural Experiment Station Publication # D-08149-13-87, supported by State and U. S. Hatch Act funds.

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