Nitrogen budget of *Callosobruchus maculatus* larva developing in different host seed species

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Abstract. Studies on effect of seed species and their interacting influence on food utilization of *Callosobruchus maculatus* clearly pointed out that rates and efficiencies of nitrogen utilization are determined by the composition of the seed also. Of the 3 species of seeds tested, *Vigna unguiculata* has significantly more nitrogen and water per unit weight than the other two seeds, *Dolichos lab lab* and *Phaseolus radiatus*. With increasing nitrogen content of the seed, rates of feeding, assimilation and metabolism decreased. On the other hand efficiencies of assimilation and net production increased. Nitrogen assimilation efficiency of *Callosobruchus maculatus* ranged from 76·6% in *Dolichos lab lab* to 85·8% in *Vigna unguiculata*. The efficiency of accumulation of assimilated nitrogen was around 82%. The less nutritious *Dolichos lab lab* contributed more to the observed variance in the efficiencies of assimilation and production. The low efficiencies may be attributed to the presence of protease inhibitors.

Keywords. Nitrogen budget; *Callosobruchus maculatus*; *Dolichos lab lab*; *Phaseolus radiatus*; *Vigna unguiculata*.

1. Introduction

The pattern of food utilization and energy allocation to reproduction in insects is significantly influenced by humidity (Pingale 1976), food quality (Singh and Krishna 1980), temperature (White and Sinha 1981), life style (Cairns 1982) and interaction of one or more of these factors (Muthukrishnan and Pandian 1984). Scriber and Slansky (1981) showed that nitrogen and water content of food may significantly influence the assimilation efficiency of lepidopteran insects. The present observation pertains to the nitrogen budget of a granivorous insect *Callosobruchus maculatus* (F). feeding on different host seed species.

2. Materials and methods

2.1 *Estimation of nitrogen content*

Nitrogen budget of *C. maculatus* was studied as functions of host seed species, *Vigna unguiculata*, *Phaseolus radiatus* and *Dolichos lab lab*. Seeds were taken in 5 cm dia petridish. Newly emerged adults were allowed to oviposit on the seeds. Subsequent to oviposition, seeds with single egg were separated, weighed individually, numbered with marker and transferred to glass vials. Ten replicates of 200 seeds containing one egg each were maintained. Simultaneously, 100 seeds of more or less the same weight but without any egg were maintained in

petridish as control. To ascertain hatching and moulting to higher instar, a minimum of 5 infested seeds from each replicate were dissected everyday. The presence of exuvia indicated moulting to the next instar. On the day of moulting to the next instar, unfed seed remains, egesta and exuvia were separated and dried. The freshly moulted larvae were weighed and dried. The dried materials were stored for nitrogen analysis.

Food consumption (*C*), egestion (*F* + *U*) and production (*P*) were estimated in terms of dry weight separately for each instar. Dry weight of the infested seed on any day of the experiment was calculated considering the water content of the uninfested control seeds. Faeces egested was estimated by collecting the faeces from each seed separately, on the day of moulting to next instar. Subtracting the dry weight of faeces for the earlier instar(s) from the total dry weight of the faeces on the day of moulting to the next instar *FU* for each instar was estimated.

Production was calculated by subtracting the dry weight of the larva at the end of each instar from that at the commencement of the instar. Exuviae were pooled, dried and weighed and the mean weight of an exuviae was calculated. For the estimations of consumption, egestion and growth of the 4 larval instars of *C. maculatus* about 1500 infested seeds were dissected. For collecting samples of faeces and insects of different life stages and for estimation of chemical composition, about 1500 seeds were dissected separately at each tested condition.

Samples of food, faeces and larvae of different stages were dried at 80°C to weight constancy. Relating the dry weight of the insect of any chosen stage to its live weight, dry matter content of the stage was estimated. All the weighings were made in a monopan balance (Sauter, West Germany) to an accuracy of 0·01 mg.

Total nitrogen content of the sample (food, faeces and insect) was estimated in a microkjeldahl apparatus following the procedure described by Umbreit *et al* (1972). Each estimation was repeated thrice and an average was taken into account.

The scheme of nitrogen budget followed is the slightly modified IBP formula (Petrusewicz and Macfadyen 1970) represented as $C = P + R + F + U$, where *C* is the total food consumed, *P* the growth, *R* the nitrogen metabolised and $F + U$ the nitrogen loss via faeces; it has been described in detail elsewhere (Muthukrishnan *et al* 1979). The rates are expressed in terms of $mg\ g^{-1}$ live insect d^{-1}. The total protein, carbohydrate and lipid content of the seeds were estimated following the methods of Lowry *et al* (1951) and Seifter and Dayton (1950) respectively.

2.2 *Statistical procedure*

Regression lines were fitted by the method of least squares. The slopes and intercepts of the regression lines are compared between different components of the food and the parameters. Significant levels were read from the tables given by Zar (1974).

3. **Results**

Nitrogen content of the food, *C. maculatus* larva and its faeces are provided in table 1. As the larva developing in *D. lab lab* doubled its larval duration and consumed more food, quantity of food nitrogen ingested by it (1·149 mg) was significantly more than that developing in the nitrogen rich *V. unguiculata* (0·528 mg) or

Table 1. Nitrogen content of host seed species, larva of *C. maculatus* and its faeces.

Material	*V. unguiculata* (mg/mg)	*P. radiatus* (mg/mg)	*D. lab lab* (mg/mg)
Host seed species	0·027 ± 0·002	0·025 ± 0·001	0·019 ± 0·003
C. maculatus larva			
I	0·151 ± 0·002	0·165 ± 0·002	0·162 ± 0·003
II	0·151 ± 0·001	0·165 ± 0·001	0·162 ± 0·002
III	0·123 ± 0·004	0·137 ± 0·003	0·235 ± 0·001
IV	0·113 ± 0·002	0·123 ± 0·004	0·225 ± 0·002
V	0·122 ± 0·001	0·132 ± 0·003	0·247 ± 0·003
Faeces (pooled)	0·022 ± 0·001	0·021 ± 0·001	0·016 ± 0·002

Values are expressed in mg dry weight.

P. radiatus (0·558 mg) (table 2). About 75–80% of the dietary nitrogen was lost through faeces and urine corresponding to the low nitrogen content of *D. lab lab*, nitrogen content of the faeces egested by the larva was also less.

Nitrogen assimilated by *C. maculatus* developing in *V. unguiculata*, *P. radiatus* and *D. lab lab* amounted to 0·453, 0·474 and 0·880 mg for the entire larval period at an overall rate of 25·94, 27·07 and 24·48 $mg\,g^{-1}\,d^{-1}$ with an overall efficiency of 85·8, 84·9 and 76·6% (table 2). Analysis of variance data show $F_{(1)2, 24} = 272\cdot24$; $F_{(1)3, 24} = 11\cdot79$; $P < 0\cdot01$ (table 3). The larva developing in *D. lab lab* accumulated almost twice more nitrogen (0·721 mg) than that developing in *V. unguiculata* (0·378 mg) and *P. radiatus* (0·384 mg) and the rate averaged to 17·3, 14·4 and 12·3 $mg\,g^{-1}\,d^{-1}$. It may be pointed out that contribution by the seed species to the total variance was more than that at the different instars $(F_{(1)2, 24} = 11\cdot3; P < 0\cdot01)$ (table 4).

4. Discussion

Chemical composition of food determines the rate of utilization. Importance of nitrogen in insect-plant interaction has been recently reviewed by McNeill and Southwood (1978) and Mattson (1980). Water has been shown to be an equally important factor in regulating food consumption and growth of insects (Scriber and Feeny 1979). Overall feeding performance of insects is determined by the positive and negative influences of the constituents of the food (Bernays and Simpson 1982).

During the larval period, holometabolous insects accumulate energy in the form of lipid to tide over the non-feeding pupal and adult stages (Gilbert 1964; Waldbauer 1968). Accumulation of nitrogen is essential for egg production during adult stage. Therefore, with increasing nitrogen, fat, energy and water contents the production rate of *C. maculatus* increased (figure 1). Similar results have been reported for the larvae of *Pericallia ricini* by Krishnan (1984). *C. maculatus* which do not feed during the adult stage adopt the following strategies like other lepidopterans (i) shortening of the mortality prone larval duration (Muthukrishnan and Pandian 1984) and (ii) increase in the rate and efficiency of food consumption and conversion during the adult stage (Waldbauer 1968; Pandian 1973).

Assimilation efficiency of *C. maculatus* bears a highly significant positive

Table 2. Effect of seed species on the nitrogen budget of *C. maculatus* developing in *V. unguiculata*, *P. radiàtus* and *D. lab lab*.

Parameters	V. unguiculata	P. radiatus	D. lab lab
Duration (D)	16·0	17·5	30·0
NC[a]	0·528	0·558	1·149
NFu[a]	0·075	0·084	0·269
NA[a]	0·453	0·474	0·880
NP[a]	0·378	0·384	0·721
Residual[a]	0·074	0·077	0·159
NCr[b]	30·35	31·65	31·62
NAr[b]	25·94	27·07	24·48
NPr[b]	17·29	14·38	12·31
NAe (%)	85·79	84·95	76·60
N Pe$_1$ (%)	71·60	68·82	62·75
N Pe$_2$ (%)	83·44	81·01	81·93

[a]mg individual^{-1}; [b]mg g^{-1} d^{-1}.

Table 3. Analysis of variance for the data on nitrogen assimilation efficiency of *C. maculatus* as a function of life stage and host seed species.

Life stage	Host seed		
	V. unguiculata	P. radiatus	D. lab lab
Instars			
I	85·7 ± 1·1	86·2 ± 2·1	78·2 ± 1·5
II	85·7 ± 1·4	85·5 ± 2·2	77·6 ± 1·2
III	83·9 ± 1·0	83·4 ± 1·7	75·1 ± 1·3
IV	85·1 ± 0·9	85·1 ± 0·9	76·9 ± 0·9

Analysis of variance

Source	SS	df	MS	F	P
Total	564·62	35	—	—	—
Between host	509·09	2	254·55	272·24	0·01
Between instar	33·08	3	11·03	11·79	0·01
Interaction	3·11	6	0·519	0·555	0·05
Error	22·45	24	0·935	—	—

correlation with nitrogen, energy and water contents of the seed species (figure 1). In a series of publication Pandian and Marian (1985) also reported a positive correlation between assimilation efficiency and food nitrogen for aquatic insects, lepidopterous larvae, polychaetes, fishes and reptiles. A low food nitrogen and water enhance feeding rate and suppress the assimilation efficiency of the incumbent (Lawton 1971; Muthukrishnan and Pandian 1984). Hence, while developing in nitrogen and water rich *V. unguiculata*, *C. maculatus* displays'a low feeding rate and a high assimilation efficiency. The low efficiency of *C. maculatus* in *D. lab lab* may be due to the presence of saponins which interfere with the process of digestion (Applebaum and Guez 1972; Janzen *et al* 1976).

The positive correlation between net production efficiency and nitrogen may be due to the fact that it has to process more food to acquire nitrogen sufficient
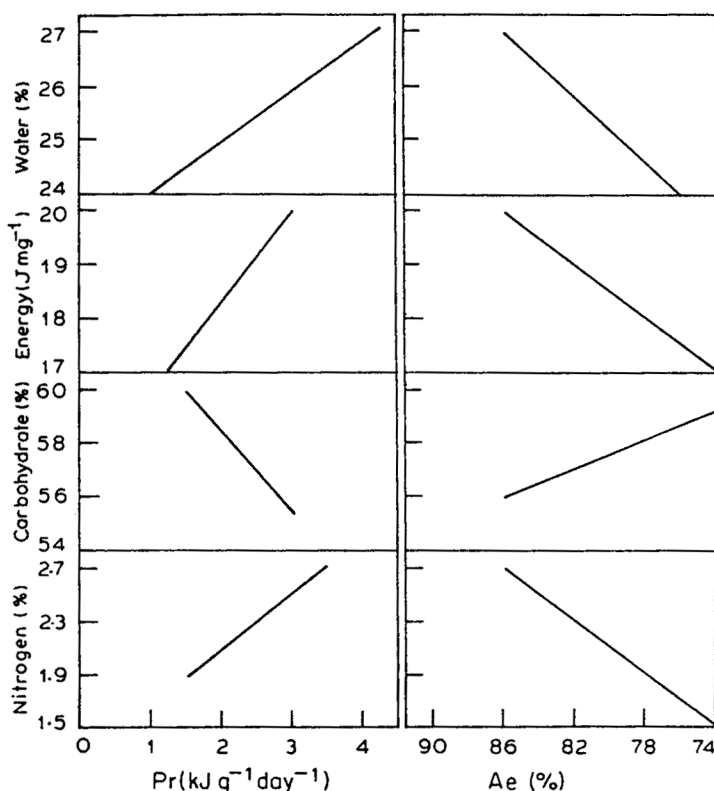
Figure 1. Effects of nitrogen (N), carbohydrate (C), energy (E) and water (W) and contents of grains on rates of production and assimilation in *C. maculatus*.

$$Y = -1.850 + 1.806 \text{ N}; \quad r = 0.992$$
$$\text{Pr} \quad Y = -25.570 + (-0.405)\text{C}; \quad r = 0.999$$
$$Y = -8.570 + 0.590 \text{ E}; \quad r = 0.998$$
$$Y = -6.800 + 0.363 \text{ W}; \quad r = 0.998$$

$$Y = 44.07 + 15.79 \text{ N}; \quad r = 0.989$$
$$\text{Ae} \quad Y = 281.86 + (-3.50)\text{C}; \quad r = -0.992$$
$$Y = -12.91 + 5.06 \text{ E}; \quad r = 0.976$$
$$Y = 4.14 + 3.04 \text{ W}; \quad r = 0.944$$

enough to complete development successfully. The dependence of rate and efficiency of food consumption and utilization on nitrogen content of the seeds is also explained by the significant correlation between developmental rate and nitrogen content. With increasing nitrogen content of the seed, development is accelerated. Larval development in insects involves periodical moulting and synthesis of new cuticle. Availability of nitrogen regulates the process of moulting and formation of cuticle (Gilbert 1964; Wigglesworth 1972). Therefore, development rate of *C. maculatus* feeding in low nitrogen containing *D. lab lab* is slower than that developing in *V. unguiculata* or *P. radiatus*. The gross nitrogen accumulation efficiency (NPe₁) of *C. maculatus* ranges from 62·8% for that developing in *D. lab lab* to 68·8% and 71·6% for those developing in *P. radiatus* and *V. unguiculata*. Food utilization by *C. maculatus* is determined by the nitrogen content of the seed.

Table 4. Analysis of variance for the data on net nitrogen production efficiency (N Pe$_2$) of *C. maculatus* as function of life stages and host seed species.

Life stage	Host seed		
	V. unguiculata	*P. radiatus*	*D. lab lab*
Instars			
I	48·2 ± 1·3	43·1 ± 2·6	26·7 ± 2·2
II	68·6 ± 2·2	56·5 ± 2·1	28·6 ± 1·6
III	53·2 ± 1·9	52·1 ± 1·9	59·4 ± 2·1
IV	86·5 ± 2·4	82·9 ± 1·8	74·9 ± 2·2

Analysis of variance					
Source	SS	df	MS	F	P
Total	12208·69	35	—	—	—
Between host	1823·47	2	911·73	11·30	0·01
Between instar	8494·07	3	2816·35	34·91	0·01
Interaction	1918·43	6	319·73	03·96	0·01
Error	1936·15	24	80·67	—	—

V. unguiculata seed was preferred and more suitable than *P. radiatus* or *D. lab lab* seed for *C. maculatus* because this food source shortened the larval duration, facilitated higher production and enhanced population growth.

Acknowledgement

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