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What Can Nutritional Indices Tell Us about Gypsy Moth Larvae (*Lymantria dispar* L.)?

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SUMMARY

In the submitted work, important factors for planning out experiments for studying nutritional indices, whose choice may affect research results, and opportunities for using the indices of feeding to study the ecology of species are evaluated. The dependence of nutritional indices on great many factors and significant variation in their absolute values over different years make possible their research only as part of a complex issue together with population factors and external conditions. A careful comparison of results from different studies is made.

Keywords: Gypsy moth; Nutritional indices; Trans-Urals and Lower Volga populations; Population structure; Experimental method

INTRODUCTION

In the classical works devoted to studying the biology and ecology of gypsy moth, surveys and data from natural conditions were one of the basic methods of studying gypsy moth (Il'inskiy, 1959). Later studies of a growing number of morphological and physiological indices in experimental laboratories allowed us to considerably expand the volume of our knowledge about gypsy moth.

Studies of nutritional connections of gypsy moth have involved many researchers, first of all for drawing up a list of preferred host species and for estimation of the quantity of food consumed by larvae with the purpose of forecasting damage which gypsy moth is able to cause to forest plantings (Il'inskii, 1959; Kozhanchikov, 1950). Calculation of the basic nutritional indices – approximate digestibility of food (AD), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) – has opened new opportunities for research of the power of feeding.

Technical work during studies of nutritional indices deals with distinctions caused by the following components: food eaten by larvae, larval density, duration of experiments and number of instars.

MATERIAL AND METHODS

Larvae of the gypsy moth were reared under laboratory conditions from egg masses collected from different geographical populations (Trans-Urals population Sverdlovsk Province, Kamensk-Ural'sk forestry enterprise, Pokrovskoe forest establishment; Lower Volga population Volgograd province, Tumak settlement). The eclosion of larvae and their development (individually in Petri dishes) took place in growth chambers at 27°C temperature and 60-70 % humidity on an artificial diet (Il'inykh, 1996). After the third instar, nutritional indices were calculated from data such as the weight of food consumed by insects, feces found and growth in biomass of each larva which were measured daily. The following indices were calculated, which are widely used as insect feeding characteristics – AD, ECI and ECD (Waldbauer, 1968). The sex (in pupal stage) and number of instars in larval ontogenesis were also determined. Statistical processing of results was carried out by standard methods using STATISTICA 6.0 software. Confidence intervals of the average data were defined by Student's criterion.

RESULTS AND DISCUSSION

Food for larvae

There are many works in which larval feeding indices have been studied based on eating of foliage or needles of host plants (Barbosa and Greenblatt, 1979; Vshivkova, 1983). In our opinion, the use of a natural food has time limits and allows to obtain data only about the reaction of larvae to any particular host plant under particular conditions of feeding since the structure of foliage is exposed to significant daily and seasonal fluctuations. We have shown in earlier studies that morphometric, ontogenesis and nutritional indices vary depending on conditions of gathering of foliage, i.e. whether leaves have been collected within a day or a season, once only or on a daily basis (Ponomarev et al., 2001). In working out the food incides, the lack of works in which larvae eat foliage, in our opinion, make it difficult to distinguish between an influence of seasonal dynamics on the biochemical structure of foliage on the one hand, and changes taking place in larval organisms in a process of growth on the other hand.

In studying the trophic indices calculated for larval organisms, in our opinion, the optimal method is to use artificial diet that excludes the influence of food biochemical heterogeneity. Such approach was used in some works mainly by American researchers (Lance et al., 1986; Stockhoff, 1993; Lindroth et al., 1997). In our experiments, an artificial diet developed by Il'inyh (1996) was used. The artificial diet is simple in structure and technique of preparation and allows to rear larvae successfully into the pupal stage. The results of long-term laboratory experiments have shown that artificial diet allows the researcher to get a picture that reflects real conditions in a natural population of gypsy moth. So the cultivation of larvae from an egg mass collected from a Trans-Urals population in 2005 on artificial diet has revealed some distinctions between separate micropopulations regarding the speed of larval development, morphometrics and physiological indices. These results are fully consistent with the situation in natural conditions and have been confirmed in field researches (Ponomarev and Andreeva, 2008).

The structure of the artificial diet used by American researchers (the main component is wheat grains) (Bell et al., 1981) was different from the diet in our research (where corn and soybean flour made the basic component). Therefore it is necessary to approach with care to comparing the results as differences can be caused not by population distinctions, but the structure of artificial diet.

Larval Density

Fluctuations in gypsy moth density in a natural population and the resulting change in their basic morphological and physiological indices are known to be typical throughout their development. During cultivation of larvae on artificial diet (at uniformed density) it has been shown that morphometric and nutritional indices vary in different stages of development depending on external (hydrothermal) conditions, i.e. depending on a density of natural population (Andreeva et al., 2008). Besides, the results obtained earlier by some authors experimentally confirm that some indices, including nutritional, are determined by the density of larvae during their eating of foliage or needles (Vshivkova, 1982; Ponomarev et al., 2009). We have confirmed these data during cultivation of larvae on the IRS, and it was shown that distinctions between single and group larvae regarding the complex of nutritional indices had different force during depression and increase in population size.

The common laws of increase in ECI and ECD among group larvae irrespective of the food they eat (artificial diet or natural food) (Andreeva, 2009) were confirmed.

It is necessary to take into account that the density of cultivation of larvae during an experiment should also be defined by the purpose of research. In most parts of the work, the author used individually reared larvae. The main advantage of this method, in comparison with group breeding of larvae, is that individual cultivation during all (larval) phase allows us to monitor each gypsy moth individual, determining such indices throughout the development of caterpillars (larvae) and all separate larval instars, as well as the number of larval instars in ontogenesis, sex, phenotype, nutritional indices for each instar and for the entire period of feeding. We can further classify data based on these indices.

Duration of experiments

Duration of experiments may be some days, or the supervisions carry out during separate instars or all period of development of the larvae phase (Baranchikov, 1987; Vshikova, 1983). It is known that during the gain of weight of larvae, food intake and excretion occur irregularly. So, it is direct after moulting into a next instar that a lot of food intake occurs, but insignificant feces excretion. At the end of the instars stage, excrement is found while food intake is insignificant or terminated. Accordingly, nutritional indices of feeding vary in terms of experiment duration. Figure 1 (a, b, c) shows nutritional indices of the last instar of females having six instars in their ontogenesis. Besides the curves in diagrams that are showing average and cumulative values there is also a curve constructed from those values, designed for each day. The diagrams show that feeding indices per days display maximal values at the beginning of instars and minimal at the end of them. On the last day before cocooning, even negative values of ECI and ECD were marked. It shows a loss of weight and processes occurring in the larva before pupation, especially the cleaning of intestines and water discharge.

The AD index is characterized by higher average values and, as a rule, a greater dispersion in comparison with the ECI and ECD. The maximal, minimal and average values of the AD were 63.5, 26.7 and 37.2, respectively; while the respective ECI values were 31.1, -7.1 and 6.9, and ECD 49.4, -26.7 and 18.6 .

From the cumulative diagram constructed by addition of daily gain in larval weight, food intake and excrement, it is evident that, using one- two- and three-day data, we acquired overestimated AD values compared with the average for the instar. And although they were further located close to the average (in the middle of the stage, variation of the values for 3-day AD was 39.7, on the average 37.2), the final value, as for the ECI and ECD, turns out only on the last day. For the last two indices only data for all instars allow the construction of values close to the average, otherwise they also are considerably overestimated.

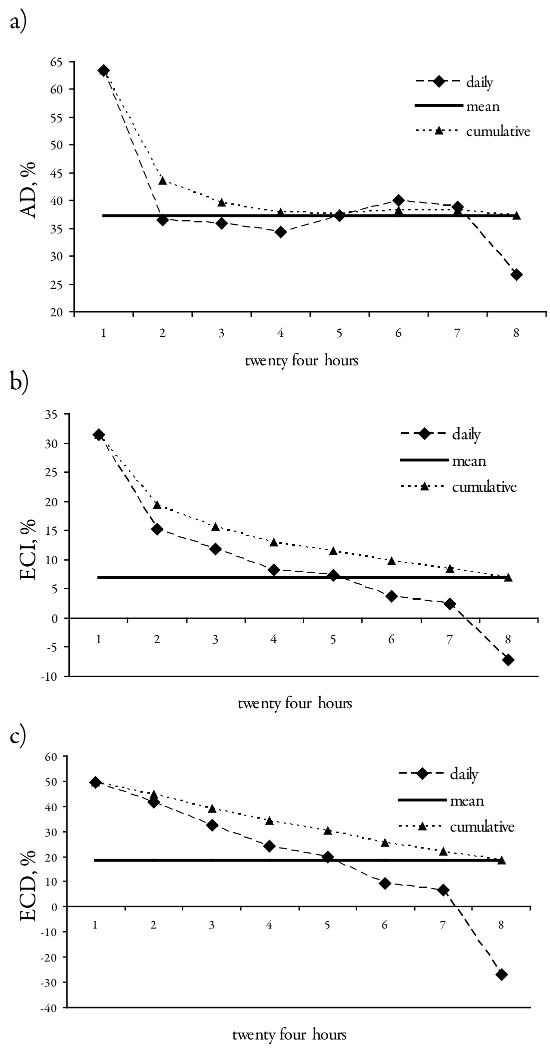

Figure 1. Nutritional indices AD (a), ECI (b) and ECD (c) for female six-instars larvae. Trans-Urals population, egg mass, 2002

Sample size of individuals is also important. Beginning with the second gypsy moth instar, distinctions were observed in larval gain, depending on the overall number of instars in larval ontogenesis. In larval instars 3-4, powerful energy input brings also the future sex of the individuals. Greater weight gain (Table 1) and quantity of consumed food and excrement were found for both male and female individuals with less additional instars. The influence of the sex grew in the process of larval growth.

On the other hand, data showing a shorter period of development of middle instars were obtained from a comparison with the first, penultimate and last instars (Edel'man, 1956; Kireeva, 1983). It is obvious that carrying out of the experiments during instars of shorter duration, for example 3-4 instars, will ensure a lower probability of data distortion and minimize a mistake for the average on the one hand, and require a smaller number of individuals on the other. In our opinion the number of individuals should be increased with instars, so as to have no less than 30-50 individuals at 1-4 instars and no less than 100-120 in more advanced instars. It is connected with an increase in variability of the indices for those instars.

Using the described method – individual rearing of larvae, artificial diet and breeding at constant temperature and humidity – the author received the following results. It was established that a significant influence is rendered on nutritional indices by sexual, phenotypic and age structure, as well as the host species which the parental generation of larvae had eaten. It was shown that the distinctions found between micropopulations from different host species inside one population are more significant than between micropopulations from one host plant but from different populations (Andreeva, 2002). In our opinion, it leaves an uncertainty regarding the possibility of using nutritional indices for finding distinctions between populations, but leaves some opportunity for their use only in view of their development cycle and hydrothermal conditions during the period of feeding of parental generation in natural conditions.

Long-term experiments for studying nutritional indices may initially cause some confusion to the researcher. It appears that in all investigated geographical populations of gypsy moth (one micropopulation and one species) the values of indices of growth, development

Table 1. Growth and food intake weight at different instars, nutritional indices of the third instar of larvae with different number of instars in their ontogenesis. Trans-Urals population, egg mass, 2002

Parameters	Males 5 instars, *n*=27	Males 6 instars, *n*=8	Females 5 instars, *n*=8	Females 6 instars, n=63	Females 7 instars, *n*=3
Growth, mg					
II instar	14.4 ± 0.9	8.8 ± 0.4	19.8 ± 1.0	12.5 ± 0.6	9.8 ± 1.1
III instar	47.8 ± 2.6a	31.5 ± 3.4b	79.6 ± 4.8c	42.5 ± 1.4a	22.6 ± 2.7d
IV instar	140.9 ± 6.0a	51.7 ± 8.0b	308.1 ± 22.8c	123.3 ± 4.0d	44.4 ± 9.7b
V instar	355.3 ± 23.4a	127.9 ± 12.4b	1161.0 ± 99.3c	372.3 ± 12.1a	120.3 ± 3.8b
VI instar	–	305.3 ± 34.1	–	1201.0± 40.0	307.0 ± 14.3
VII instar	–	–	–	–	929.3 ± 197.8
In total	544.0 ± 28.9a	516.3 ± 49.7b	1549.0 ± 126.4c	1737.0 ± 51.3c	1424.0 ± 194.0c
Food intake weight, mg					
III instar	71.1 ± 3.1a	53.3 ± 4.9b	113.6 ± 6.9c	64.0 ± 2.1a	45.7 ± 6.0b
IV instar	215.0 ± 9.0a	105.1 ± 18.3b	441.6 ± 35.9c	172.3 ± 6.0d	90.6 ± 8.7b
V instar	717.8 ± 46.9a	192.8 ± 15.8b	2554.2 ± 268.2c	513.9 ± 15.3d	195.1 ± 12.6b
VI instar	–	656.2 ± 63.2	–	2758.0 ± 106	477.4 ± 53.8
VII instar	–	–	–	–	2029.3 ± 325.1
Total	1003.9 ± 54.1a	1007.5 ± 84.3a	3109.4 ± 307.8b	3505.8 ± 120b	2838.2 ± 299.5b
III instars					
AD	41.5 ± 2.1a	47.4 ± 2.3a	31.7 ± 1.7b	39.8 ± 1.1ab	53.5 ± 6.6ac
ECI	10.8 ± 0.4a	9.5 ± 0.4b	11.2 ± 0.4a	10.6 ± 0.3a	7.9 ± 1.0b
ECD	25.9 ± 2.5a	19.9 ± 1.5b	35.4 ± 2.4c	26.7 ± 2.5a	14.8 ± 4.3b

Significant difference ($P<0.05$) between variants inside a line is designated by different letters and feeding not only vary considerably in different years, but also show very strong differences within each year (Table 2). It can be explained by the results variance received by different researchers, especially when carrying out experiments once.

Caterpillar larvae reacted differently to the external conditions during development of their parental generation, except in different phases of the outbreak. Researches of the Trans-Ural and Lower Volga populations have shown that, during depression, the influence of hydrothermal conditions in host forest stands on the whole complex of indices, including nutritional, was significantly growing. It occurred despite the distinctions between the two researched populations regarding host plants, forest and hydrothermal conditions. In the period of increased density of larvae and in the first year of outbreak of gypsy moth a sharp decrease in reaction of the analyzed indices to changed external conditions was marked (Andreeva et al., 2008).

Thus, research of a single population during several years allows us to include in the analysis a study the influence of external factors. First of all, it can be the forest (in analyses of larval indices from different micropopulations), as well as hydrothermal conditions during the parental generation of larvae (precipitation, temperature, cold periods during larval development) (Andreeva et al., 2008; Ponomarev et al., 2009).

CONCLUSIONS

Summing up the data, it is possible to make the following conclusions:

1. In order to get reliable results from studying nutritional indices of gypsy moth larvae (and other phytophagous insects) careful planning of experiments is necessary.

2. The research results show that nutritional indices are a complex parameter reflecting the features of growth and development of gypsy moth. The indices of feeding are influenced by internal factors (population structure) on the one hand, and external conditions of development on the other. Besides, it is important to remember that population structure is mobile, varies annually and research results will be different in different years too.

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Table 2. Nutritional indices of the last instar (fifth for males, sixth for females) of gypsy moth larvae. Trans-Urals and Lower Volga populations

Year of egg mass collection	Males			Females		
	AD	ECI	ECD	AD	ECI	ECD
Trans-Urals population						
1998	33.5±2.7	8.7±1.7	28.1±6.5	35.9±2.4	9.5±0.7	26.4±1.4
1999	37.8±0.7	8.0±0.1	21.6±0.7	33.9±1.0	7.9±0.2	24.2±1.3
2000	39.5±1.2	7.4±0.1	18.7±1.6	37.3±1.0	6.9±0.1	18.4±0.7
2001	37.7±1.0	11.8±0.3	31.2±1.0	38.0±1.1	7.0±0.2	18.4±0.7
2002	41.2±0.7	7.9±0.3	19.2±0.8	38.2±0.6	7.0±0.1	18.2±0.3
2003	37.6±0.7	7.8±0.2	20.7±0.7	36.6±0.8	6.1±0.2	16.7±0.6
2004	37.7±1.4	8.1±0.9	21.5±3.0	36.3±0.4	7.1±0.1	19.5±0.3
2005	41.6±0.6	9.1±0.1	21.9±0.4	40.8±0.8	9.3±0.3	22.7±1.1
Lower Volga population						
2001	43.3±1.6	6.7±0.3	15.9±1.0	41.9±1.1	6.8±0.3	16.3±0.6
2002	41.3±0.8	7.7±0.2	18.6±0.4	40.2±0.8	6.8±0.1	17.0±0.5
2003	42.0±1.3	6.9±0.2	16.6±1.0	37.5±0.9	6.7±0.2	17.7±0.6
2004	41.1±0.8	7.7±0.1	18.6±0.4	34.7±0.5	6.7±0.1	19.3±0.4

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Šta nam pokazuju indeksi ishrane o larvama gubara (*Lymantria dispar* L.)?

REZIME

U priloženom radu ispitani su najpre faktori neophodni za planiranje eksperimenata za proučavanje indeksa ishrane, čiji izbor može uticati na rezultate istraživanja, kao i mogućnosti korišćenja indeksa ishrane u proučavanju ekologije vrste. Zavisnost indeksa ishrane od većeg broja faktora i značajno variranje apsolutnih vrednosti u različitim godinama omogućavaju istraživanje samo u sklopu populacionih faktora i uslova spoljašnje sredine. Sa pažnjom je izvršeno poređenje rezultata iz različitih istraživanja.

Ključne reči: Gubar; indeksi ishrane; populacije Trans-Ural i Donja Volga; struktura populacije; eksperimentalna metoda