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ON THE EFFECT OF MICROSPORIDIOSIS ON HIBERNATING PUPAE OF NOCTUIDS

To predict the numbers of pests and to organize proper control over the pests, it is necessary to know their winter mortality and its causes. The survival of insects under conditions of a rather long and severe winter characteristic of the temperate zone is ensured by dormancy, a state resulting as a special adaptation to climatic conditions. Several types of winter dormancy can be met with in nature. The insects that hibernate under the snow cover, including the pupae of noctuids, as a rule enter the state of diapause with the approach of winter, this state being induced before the end of the vegetative period (Данилевский, 1961). Diapause is characterized by an intense restraint of metabolism a three- to five-fold diminution of oxygen consumption (Schneiderman, Williams, 1953; Ушатинская, 1957; Chino, 1958), which is accompanied by dehydration, an increase in the ion concentration of the haemolymph and several other processes eventually leading to a general increase in the resistance to unfavourable environmental factors. An extremely significant circumstance results directly from the physiological mechanism of diapause: the more intense the diapause, the greater the power of resistance in winter. The latter implies the resisting power of an organism throughout the dormancy season, i.e. during autumn, winter and spring.

All the factors that inhibit the induction of diapause or that prevent diapause from becoming more intense, at the same time reduce the survival of insects in winter. Evidence is available in the literature of the fact that various factors (such as overcrowding, food of inferior quality, several bacterial and fungal diseases) may inhibit the normal development of diapause (Bonnemaison, 1962; Мериээ, 1972; Самедов, Абдурахманов, 1971). It is well known that parasitic unicellular organisms belonging to the order of *Microsporidia* produce changes in the physiological state of host insects. The effect of microsporidiosis (the disease induced by *Microsporidia*) on overwintering insects has not been studied so far. Since diapause is formed by hormonal and neurohormonal mechanisms, the disturbance of the hormonal balance by protozoa may impede the development of the diapause. The present paper discusses the effect of the microsporidian *Plistophora schubergi* Zwölfer (*Microsporidia, Nosematidae*) on the pupal diapause in four species of noctuids: *Barathra brassicae* L., *Mamestra dissimilis* Kn., *Mamestra persicariae* L. and *Mamestra pisi* L.

Material and methods

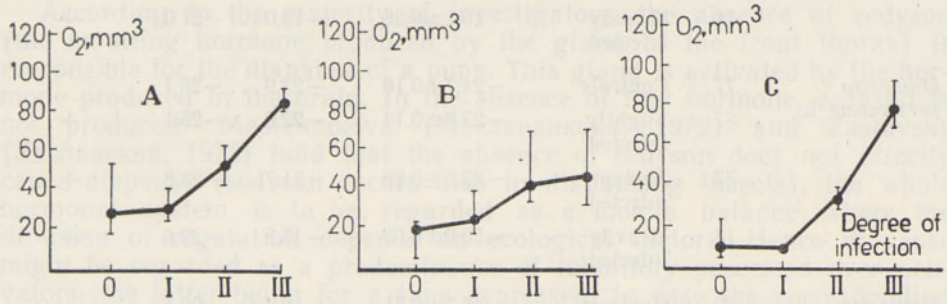
The moths needed for experiments were caught by means of light traps and were placed in pairs into 1-litre jars where they were fed on 5% sucrose solution. The eggs laid were kept in Petri dishes at room temperature. The hatched caterpillars were placed in rearing jars (likewise 1-litre glass jars) where they were reared at a day-length of 12.5 hr at a temperature of 21 °C. Third-instar larvae were infected with the spores of the microsporidian *P. schubergi* Zwölfer, which were placed on the leaves of food plants. The titre of the inoculum ranged from 1×10^4 to 1×10^6 spores per individual. Moist peat or sand was placed in the jars for purposes of pupation. The pupae of all the experiments were kept under outdoor conditions from where they were brought into the laboratory immediately before determination. The cold-hardiness of diapausing pupae was determined thermoelectrically by way of the determination of the supercooling points, using a copper-constantan thermocouple and the method which has been repeatedly described (Горышин, 1966; Куузик, 1971). The intensity of respiration of the hibernating pupae was determined manometrically by means of a Warburg device: the oxygen amount consumed in an hour per mm^3 per gramme of live weight was fixed at 20°.

Results and discussion

When assessing the intensity of the diapause, one may proceed from various criteria. In the opinion of some investigators (Lees, 1968; Данилевский, 1961), the rate of reactivation is the most universal indicator to be used in comparing different insect species and different developmental stages among themselves. The species of moths studied by us emerge and start flying about in June and July, which, taken by itself, should indicate a rather long period of reactivation and thus a rather intense diapause. The reactivation rate is correlated with metabolism: reactivation proceeds faster at a higher level of metabolism. The healthy pupae of the noctuids studied had a relatively low level of respiration (20 to 40 mm^3 O_2 per gramme of live weight per hour), which also points to a deep diapause of these species.

The intensity of aerobic respiration is regarded as another criterion of the intensity of the diapause, since it reflects the intensity of both growth and developmental processes. At the same time it must be noted that the intensity of the diapause of different species and different stages can be compared proceeding from the rate of the decrease in the intensity of oxygen consumption in passing from the active state into the dormant one. Compared with active stages, respiration in the state of diapause decreases many times. In our experiments the intensity of respiration in the diapausing pupae of *Mamestra pisi* and *Barathra brassicae* decreased ten-fold as compared with their active state. These data testify to a deep diapause. Differences in the intensity of respiration, however, do not always serve as a criterion of the depth of the diapause since there are diapausing species which simultaneously preserve the activity of movement and a high oxygen consumption. Nevertheless, this criterion may serve as a sure basis for the comparison of the intensity of the diapause in different individuals of one and the same species and at the identical developmental stage. In our experiments the intensity of the diapause was compared in healthy pupae of *B. brassicae*, *M. dissimilis* and *M. pisi* as well as in pupae infected with the microsporidian *P. schubergi*. To be able to establish the dependence of the respiratory level on the degree of infection with greater accuracy, the whole material was analyzed microscopically after determinations. The individuals that

had been infected with other diseases (viruses, bacteria, etc.) were not taken into account, although the level of their respiration was markedly higher than that of the healthy ones. According to the degree of infection, we grouped the pupae into the following variants: controls, slightly infected, medium-infected and heavily infected ones. The respiratory level of individual pupae is presented in the Figure (A, B and C). Such a distribution of the experimental material revealed strikingly the relationship between the degree of infection and the intensity of respiration. In the case of intense infection, the respiration of the infected pupae exceeded the respiratory rate of the healthy ones up to ten times, thus approaching the level of the respiration of developing pupae.



Dependence of the intensity of respiration in diapausing pupae upon the degree of infection. A — *Mamestra pisi* L.,
B — *Barathra brassica* L., C — *Mamestra dissimilis* Kn.
O — Controls; I — Slightly infected; II — Medium infected; III — Heavily infected.

Classifications of the physiological types of dormancies do not contain cold-hardiness as an independent criterion since there is no regular correlation between the intensity of dormancy and cold-hardiness. Proceeding, however, from the dynamics of the physiological indicators of dormancy of a certain overwintering stage of a species, changes in the supercooling points may reflect metabolic shifts during hibernation. An increase in the ability of supercooling results practically from the deepening of dormancy. Although the ability of supercooling is formed by various physiological processes, its biochemical essence is the same. There are many hydrophilous colloids in the haemolymph which bind the water molecules. "Bound" water freezes at a temperature which is considerably lower than the usual one, and for this reason the ability to supercool increases. This can be determined by way of the supercooling points, which practically is the freezing temperature of an insect. The supercooling points of different stages of infection of *B. brassicae, M. persicariae* and *M. dissimilis* and those of healthy diapausing pupae are presented in the Table. It appeared that in insects suffering from microsporidiosis the ability of supercooling (hence also cold-hardiness) decreased, the stage of infection being inverse to cold-hardiness. It is peculiar that in the case of an intense infection the average cold-hardiness of the pupae was reduced by 4°, and in individual cases even up to 10°. In the case of average stages of infection cold-hardiness decreased by 2°.

Thus it appeared from our laboratory experiments that microsporidiosis affects the regulatory mechanisms, as a result of which the diapause is weakened. The higher than normal intensity of respiration in diapausing pupae points to the fact that metabolism is less blocked. In the period preparatory for diapause, the reserve substances (such as

Dependence of supercooling points of diapausing pupae of some noctuids upon the degree of infection

Species	N	Treatment	Average supercooling points M±m	Limits of variance	Significance level 0.01
Barathra brassicae L.	45	Controls	−22.0±0.17	−20.0 . . . −23.5	
	15	Slightly infected	−21.3±1.85	−19.0 . . . −23.5	−
	16	Medium infected	−20.0±0.26	−19.2 . . . −20.5	+
	18	Heavily infected	−19.1±0.26	−18.0 . . . −21.0	+
Mamestra persicariae L.	77	Controls	−24.7±0.18	−23.9 . . . −25.1	
	87	Slightly infected	−23.8±0.14	−22.8 . . . −25.1	+
	32	Medium infected	−22.6±0.23	−21.7 . . . −23.8	+
	14	Heavily infected	−20.0±0.68	−15.8 . . . −22.6	+
Mamestra dissimilis Kn.	30	Controls	−25.3±0.71	−18.4 . . . −26.5	
	30	Heavily infected	−21.6±0.93	−16.0 . . . −25.0	+

glycogen, proteins, etc.) in infected pupae are in short supply or are altogether lacking, the pupae suffering from microsporidiosis face winter unadequately prepared, which increases winter mortality. Unhealthy insects consume the insufficient food supplies quickly on account of a higher metabolic rate and either perish from exhaustion or may produce totally barren offspring, sometimes underdeveloped moths. All this could be seen in the experiments which had been given higher infective doses. The decrease in cold-hardiness also indicates the fact that microsporidiosis produces disturbances in the diapausal mechanism, as a result of which the diapause remains superficial and cannot become as intense as under normal conditions. There arises the question: which link in the diapausal mechanisms is actually affected by microsporidiosis? Undoubtedly, one reason is an anomaly in the induction of the diapause. The larval stage of infected noctuids in our experiment was considerably lengthened. Since photoperiodic sensitivity may differ in different instars, there may occur a shift in the synchronization of instars with the critical photoperiod (i. e. the length of day which sets off the diapausal mechanism). There are also data available in the literature on the circumstance that in unhealthy insects photoperiodic sensitivity is reduced (Исси, Масленникова, 1963). Many investigators have reported evidence that microsporidia produce a substance in the host insects which in its action resembles the juvenile hormone (Fisher, Sanborn, 1962a, b; 1964; Новак, 1972; Приданцева, Драбкина, Цизин, 1971). Thus they directly influence the hormonal system of the insect host. The specificity of the juvenile hormone produced by corpora allata lies in the fact that it inhibits metamorphosis. Hence an excess amount of the juvenile hormone in caterpillars is responsible for the circumstance that they stay in the same developmental stage. In the early embryonic period the titre of the

juvenile hormone is low, but the growth of larvae without its high concentration is unthinkable. By the moulting time of larvae its titre is somewhat reduced, but not below the critical level. During pupation and moulting its level falls steeply. Due to the analogue of the juvenile hormone produced by microsporidia, the moulting of caterpillars has been rendered more difficult, which has often been noticed with insects suffering from microsporidiosis. Our experiments showed that a critical exceeding of the titre may also occur in pupation. The passage from one instar into another was rendered more difficult, and only a part of the caterpillars pupated. Others died in pupation or yielded abnormal pupae that died in the first period of overwintering. Thus we may suppose that microsporidia disturb the hormonal balance of the insects.

According to the majority of investigators, the absence of ecdyson (the moulting hormone produced by the gland of the front thorax) is responsible for the diapause of a pupa. This gland is activated by the hormone produced in the brain. In the absence of that hormone, ecdyson is not produced. Maslennikova (Масленникова, 1972) and Zaslavsky (Заславский, 1972) hold that the absence of ecdyson does not directly cause diapause (ecdyson occurs also in diapausing insects), the whole hormonal system is to be regarded as a mobile balance where the direction of regulation depends on ecological factors. Hence diapause might be regarded as a predominance of inhibitory processes over activators, the latter being for a time depressed. In case the corresponding photoperiod brings about prevalence of activators over inhibitors, development proceeds without a diapause. Consequently, inhibitory and activating processes proceeding simultaneously are in the most unstable equilibrium at critical titres. The minimum predominance of only one component is sufficient to give the process a reverse direction. The disturbed metamorphosis of insects with microsporidiosis and a less intense diapause can be accounted for by the conceptions of Maslennikova and Zaslavsky.

The action of microsporidia on the reduction of cold-hardiness in insects has also to be considered from the biophysical aspect of the freezing of the haemolymph. Salt (1950, 1953, 1958, 1966) states that nucleating agents are needed for the freezing of the supercooled body fluids, while the freezing rate depends on the amount of nuclei, the rate of the formation of crystals during the crystallization process and the duration of the action of temperature. When the haemolymph freezes, hibernating microsporidia themselves may serve as nucleating agents during pupation, in the spore stage. To prove this, we carried out experiments with freezing the pure physiological solution and with freezing the same solution to which spores of microsporidia had been added. From the analysis it could be concluded that the spores of microsporidia may really be regarded as nucleating agents.

In summary we may state that microsporidiosis leads to the universal stepping up of general metabolism which necessarily involves an increase in the winter mortality rate. From this it can be concluded that the employment of microsporidia in the biological control of moths opens up wider prospects than is usually thought of. Not only lethal but also sublethal and even weaker doses of spores can be effectively employed in the biological control of moths, since those measures lead metabolism to an energetic level that is dangerous or lethal to the pests, inhibiting the normal course of the preparatory period of diapause.

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MIKROSPORIDIOOSI MÕJUST TALVITUVATELE ÖÖLASENUKKUDELE

Resümee

Kapsaöölase (*Barathra brassicae* L.), ähmase aiaöölase (*Mamestra dissimilis* Kn.), mustja aiaöölase (*Mamestra persicariae* L.) ja herneöölase (*Mamestra pisi* L.) nukud talvituvad diapausiseisundis. Diapausi kui puhkeseisundi iseloomustab sügav ainevahetuse pidurdus, mis väljendub hapnikutarbe vähenemises. Diapausiga kaasnevad organismis mitmesugused ümberkorraldused, mis suurendavad tema üldist vastupanu ebasoodsatele tingimustele. Mida sügavam on diapaus, seda edukamalt suudavad putukad talvituda.

Tegurid, mis takistavad diapausi normaalset väljakujunemist, suurendavad ühtlasi talvist suremust. Üks selliseid tegureid on pisieoseliste (*Microsporidia*) seltsi kuuluva ainurakse *Plistophora schubergi* Zwölfer põhjustatud mikrosporidioos. Tootes toimelt juveniilhormooniga sarnast ainet, rikuvad pisieoselised diapausi indutseerivat hormonaalsüsteemi. Selle tagajärjel ei omanda diapaus normaalset sügavust ja ainevahetus jääb suhteliselt kõrgele tasemele. Tugevasti nakatatud öölasenukkude hapnikutarbimus on 3—5 korda suurem tervete omast ning läheneb arenevate nukkude hingamise tasemele. Keskmise nakatusastme korral on see 1—2 korda, nõrga nakatusastme korral kuni poole võrra kõrgem tervete omast. Mikrosporidioosihaigetel öölasenukkudel väheneb ka külmakindlus, mis näitab omakorda, et haigus tingib häireid diapausi mehhanismis. Näiteks väheneb tugevasti nakatunud nukkude külmakindlus tervetega võrreldes kuni 4 °C (üksikutel isenditel kuni 10°) võrra, keskmise nakatusastme korral 2° võrra ja nõrga nakatusastme korral kraadi võrra.

Tulemustest järeldub, et pisieoseliste rakendamisel öölaste bioloogilises tõrjes on märksa avaramad perspektiivid, kui tavaliselt arvatakse. Üldine ainevahetuse intensiivistumine toob paratamatult kaasa talvise suremuse suurenemise. Tõrjeks sobivad mitte ainult letaalsed, vaid ka subletaalsed ja isegi nõrgemad doosid, sest nad viivad kahjurite ainevahetuse ohtlikule või letaalsele energeetilisele tasemele, mis takistab normaalset valmistumist diapausiks.

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ВЛИЯНИЕ МИКРОСПОРИДИОЗА НА ЗИМУЮЩИЕ КУКОЛКИ СОВОК

Резюме

Куколки капустной (*Barathra brassicae* L.), отличный (*Mamestra dissimilis* Кп.), горчаковой (*Mamestra persicariae* L.) и гороховой (*Mamestra pisi* L.) совок зимуют в состоянии диапаузы. Диапауза как состояние покоя характеризуется глубоким торможением обмена веществ, которое выражается в снижении потребления кислорода. Диапауза сопровождается рядом изменений в организме, вследствии которых повышается устойчивость организма к неблагоприятным условиям среды. Чем глубже диапауза, тем успешнее переносят насекомые зиму.

Факторы, препятствующие нормальному формированию диапаузы, одновременно повышают зимнюю смертность насекомых. Выяснено, что одним из таких факторов является микроспоридиоз, вызываемый простейшим *Plistophora schubergi* Zwölfer (*Microsporidia, Nosematidae*). Продуцируя вещество, воздействие которого аналогично воздействию ювенильного гормона, простейшие нарушают индуцирующую диапаузу гормональную систему хозяина. В результате диапауза не углубляется достаточно и обмен веществ остается на сравнительно высоком уровне. Потребление кислорода сильнозараженными куколками совок в 3—5 раз (при средней зараженности — 2 раза, при слабой — 1,5 раза) превышает таковое здоровых, приближаясь к уровню дыхания развивающихся куколок. Холодостойкость куколок совок, зараженных микроспоридиозом, снижается, что свидетельствует о нарушении механизма диапаузы. Так, холодостойкость сильнозараженных куколок снизилась в среднем на 4 °C (до 10°), среднезараженных — на 2° и слабозараженных — на 1 ° по сравнению со здоровыми.

Следовательно, применение простейших в биологической борьбе с совками имеет гораздо большие перспективы, чем до сих пор предполагалось. Наибольшая интенсивность обмена веществ сопровождается повышенной смертностью насекомых в зимний период. Для биологической борьбы с совками эффективны не только летальные, но и сублетальные дозы, так как они вызывают повышение обмена веществ до энергетического уровня, который может стать для вредителя опасным или летальным.

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