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# Possible Influence of MLP Regulators in Foliage of Host Species on Invasion of Phyllophagous Insects

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**SUMMARY**

On the northern border of the Gypsy moth area (*Lymantria dispar* L., 1758), caterpillars are reorient to exogenous regulators of membrane lipid peroxidation in connection with repeated cold periods during feeding. In case of an introduction of host plants with high contents of exogenous regulators of MLP (e.g. $Fe^{2+}$) in foliage in these areas that may affect diapause duration, the boundaries of spreading and intensity of outbreaks may change.

**Keywords:** Gypsy moth; Outbreaks; Regulators of membrane lipid peroxidation; Diapause; Infestation

## INTRODUCTION

Regarding phyllophagous insect species that have periodic outbreaks, in addition to the damage caused by defoliation of host forest stands inside traditional outbreak areas the danger of further spread exist.

This danger related both to global changes in some hydrothermal conditions and changes in area of basic host plants and to the adaption of pest to new no-specific host plant species when it introduced into new regions. Precision of prediction of outbreak's abylity of these species outside their traditional areas has an important value. On the basis of such forecasts, containment measures can be planned, as well as their financing.

So in this report we would like to consider some aspects of an ability for a northwards area expansion of forest phyllophagous insect species, demonstrating it on the example of a northern Tans-Ural population of Gypsy moth (*Lymantria dispar* L., 1758). According to literature data and our own research in the northern part of the Trans-Ural population (south of Sverdlovsk region), outbreaks take place there much less often than in its central part (Chelyabinsk region). In the Chelyabinsk region outbreaks over vast areas (ten thousand hectares) are recorded once in 10-11 years, while in the south of Sverdlovsk region the interval between

outbreaks is about 20-25 years. An unprecedentedly vast outbreak on more than 60 thousand ha was registered in the period 1957-1967 only, while two next intese outbreaks that occurred in 1986-1987 and 2005-till present, covered no more than 3-5 thousand ha.

Most researchers agree that the fate of an outbreak is determined mainly by the nutritive value of foliage (needles) of host plants for larvae, and a correspondingly higher level of survival probability. Thus some authors consider that protein and carbohydrate ratio in foliage is crucial, while others believe that antifeedants are more important (in particular phenols, terpens, etc.).

We focused on one more group of substances contained in the foliage, i.e. the substances regulating Membrane Lipid Peroxidation (MLP) that are activators of free- radical processes and antioxidants.

## MATERIAL AND METHODS

Studies were carried out in the northern part of the habitat of the Trans-Ural population of Gypsy moth (in southern and central part of Sverdlovsk region, Russia). A field survey was done by standard forest pathology techniques. Route surveys and population density research were carried out at constant sample sites. Males were caught by closed pheromone-baited "milk carton" traps with dispersers containing 500 mg (+) – disparlure (made in USA).

For laboratory caterpillar rearing, annual egg masses were collected in birch plantations of the Kamensk-Ural'sk area of Sverdlovsk region near Pokrovskoe village. Rearing of Gypsy moth caterpillars in the laboratory was done in groups on constant temperature of $27^0$C and air humidity of 60%. In each variant the initial quantity of caterpillars was not less than 100 individuals. Caterpillars were fed on a standard artificial diet (AD) (Il'inykh, 1996) and an AD with added crystalline hydrate of iron sulfate ($FeSO_4$*$7H_2O$) at a concentration of 150 mg per 500 g of diet. For levelling the influence of hydrothermal conditions during development of caterpillar parental generations, parameters of caterpillars whose parental generation had fed in more or less similar conditions were analyzed. These were caterpillars from 2003 and 2008 egg masses. An analysis of carotin concentrations in caterpillar bodies and excrements was made on the third day after caterpillars completed the 5$^{th}$ instar. For the purpose of the analysis caterpillars were reared individually. Carotin extraction from caterpillars and their excrements was done in acetone. Measurements were carried out by the spectrophotometer "СФ-46" with 0.001 measurement accuracy .

Calculation was carried out using the standard formula**:** mg% = $D_{440,5}$ ′ V ′ 100000/ 213/ m, where**:** $D_{440.5}$ – indications of spectrophotometer (wave length – 440.5 nm), V – solvent volume, 213 – extraction factor, m – sample weight .

For statistical processing of data, biometric methods with elementary descriptive statistics were used in the standard software package STATISTICA 6.0.

## RESULTS

An outbreak was occurred in an area of more than 3000 ha in the south of the Sverdlovsk region in 2005. Defoliation of separate forest stands of up to 50% was detected. The density of egg masses an the autumn reached 25-30 individuals per tree. In 2006, during the period of final instar feeding and pupation (July), a sudden spell of cold weather occurred (daily average temperature did not exceed 10-12°C for two weeks,). As a result, the density of egg masses fell abruptly and in the majority of forest stands it did not exceed two individuals per tree. Long periods of cold weather (not less than 10 days with daily average temperature below 10-$12^0$C) during caterpillar feeding were recorded in 2007 and 2008. In the autumn of 2007, the density of egg masses did not change and defoliation was slight that year. In the autumn of 2008, an increase in egg mass density was recorded again. Defoliation of separate forest stands was up to 50%. In 2009 a considerable part of forest stands was defoliated up to 75-100%, and the density of egg masses in a majority of test sites in the autumn increased to 10-15 individuals per tree (Table 1).

Long-term rearing of this population of caterpillars on AD in the laboratory (since 1993) has shown a good caterpillar adaptability to this diet. Death rate seldom

**Table 1.** Change in egg masses per tree at constant test sites in the center of Gypsy moth mass reproduction from 2005 to 2009

| No. test site | Egg masses per tree | | | | |
|---|---|---|---|---|---|
| | 2005 | 2006 | 2007 | 2008 | 2009 |
| 1 | 13 | 0.5 | 1 | 3 | 11 |
| 2 | 4 | 1 | 0,5 | 2 | 6 |
| 3 | 18 | 3 | 2 | 2.5 | 15 |
| 4 | 15 | 2 | 3 | 3 | 10 |
| 5 | 22 | 2 | 1.5 | 5 | 21 |
| 6 | 15 | 1 | 1 | 4 | 19 |

exceeded 20-30%. The duration of development up to pupation averaged 40-45 days. Egg masses were collected in the same forest stand annually. However beginning with the 2006 egg masses, caterpillars either refused to feed on AD or grew extremely slowly with a lot of additional instars (the number of instars amounted up to 12). Mass cannibalism was observed: about 80% of all reared caterpillars were eaten. Very often cannibalism included only caterpillars eating the cuticle and hypodermis of victims without touching their internal contents. The cuticle has a lot of eumelanin, which is a trap of free radicals; we have supposed that cannibalism is mainly caused by a lack of endogenous catalysts of free-radical processes.

Removing the observed syndrome was possible only by either: an addition into the AD of a slight amount of $FeSO_4$*$7H_2O$ ($Fe^{+2}$ is the activator of free-radical processes) at a rate of 150 mg per 500 g of diet, or removal of ascorbic acid (antioxidant) from AD. The first method was more effective, the duration of development was notably reduced, reaching an average of 30-35 days, and death rate did not exceed 3-5%. In our case of caterpillars of the same population but with a different degree of adaptability to low temperature the nutritive value of diet depended neither on a ratio of proteins and carbohydrates, nor antifeedants (the composition of AD was constant), but on a change of the pro- vs. antioxidants ratio in the feed. According to literature data, consequences of cold stress on insects can be compensated for by endogenous and exogenous regulators of MLP. However, orientation to the endogenous regulators results in increased death rate of individuals (Ben'kovskaya, 2009). Abrupt decrease in adaptation of the caterpillars whose parental generation had survived through the long cold periods during feeding on the standard AD in that case should be caused by a considerable reduction in the amount of endogenous activators of free-radical processes.

From this standpoint, our data on the caterpillar body content showed changes (beginning of 5$^{th}$ instar) in exogenous antioxidants (carotins) both in caterpillars from the egg masses of 2008 fed on standard AD and AD supplemented with $FeSO_4$ (100% hatch after 2 months diapause) and in caterpillars from the egg masses of 2003 (up to the period of cold stress impact) at 50% hatch (2.5 months diapause) and 100% hatch (5 months diapause). At 50% hatch, caterpillars from the egg masses of 2003, as well as caterpillars from the egg masses of 2008 reared on standard AD were observed to slow down their development, have high death rate of younger instars, considerably lower caterpillar weights by the beginning of the 5$^{th}$ instar, and considerably less use of assimilated carotins (Table 2).

Table 2 shows higher concentrations of carotins in caterpillar bodies of lighter weights in the variant of 2008 on standard AD.

Taking into account the high level of negative correlation we detected between caterpillar weights and carotin contents in caterpillar bodies, amounting to -0.7 (Sevryugina and Ponomarev, 2003), with the different average values of caterpillar weights, the regression analysis of a dependence of carotin concentration on caterpillar body weight in different variants of rearing is the most indicative.

Such analysis (Figure 1) shows a high degree of this parameter identity for variants of 2003 on the standard AD at 100% hatch and of 2008 on the AD with $FeSO_4$, and also significant similarity between the 2003 vari-

**Table 2.** Parameters of caterpillar development, their weights at the beginning of 5$^{th}$ instar and assimilation of carotins from diet in different variants of rearing from the egg masses of 2003 and 2008 on AD. The egg masses from the 45$^{th}$ quarter of Pokrovskogo site, Kamensk-Uralsk district of Sverdlovsk region

| Year and variant of rearing | NN | Weight of caterpillars, mg | Carotins in caterpillar bodies, mg% | Carotins in excrement, mg% | Development up to the beginning of 5$^{th}$ instar (days) |
|---|---|---|---|---|---|
| 2003, 50% hatch | 31 | 140 ± 11.4 b | 19 ± 2.1b | 16 ± 1.7 b | 49 ± 1.5 c |
| 2003, 100% hatch | 32 | 240 ± 16.4 c | 10 ± 1.2 a | 14 ± 2.3 b | 26 ± 0.6 a |
| 2008, on AD | 33 | 64 ± 5.6 a | 46 ±4.2 c | 19 ± 1.8 b | 36 ± 0.7 b |
| 2008, on AD with $FeSO_4$ | 41 | 320 ± 16.6 d | 10 ± 0.8 a | 7 ± 1.3 a | 25 ± 0.6 a |

Significant differences ($p<0.05$) are shown by different letters

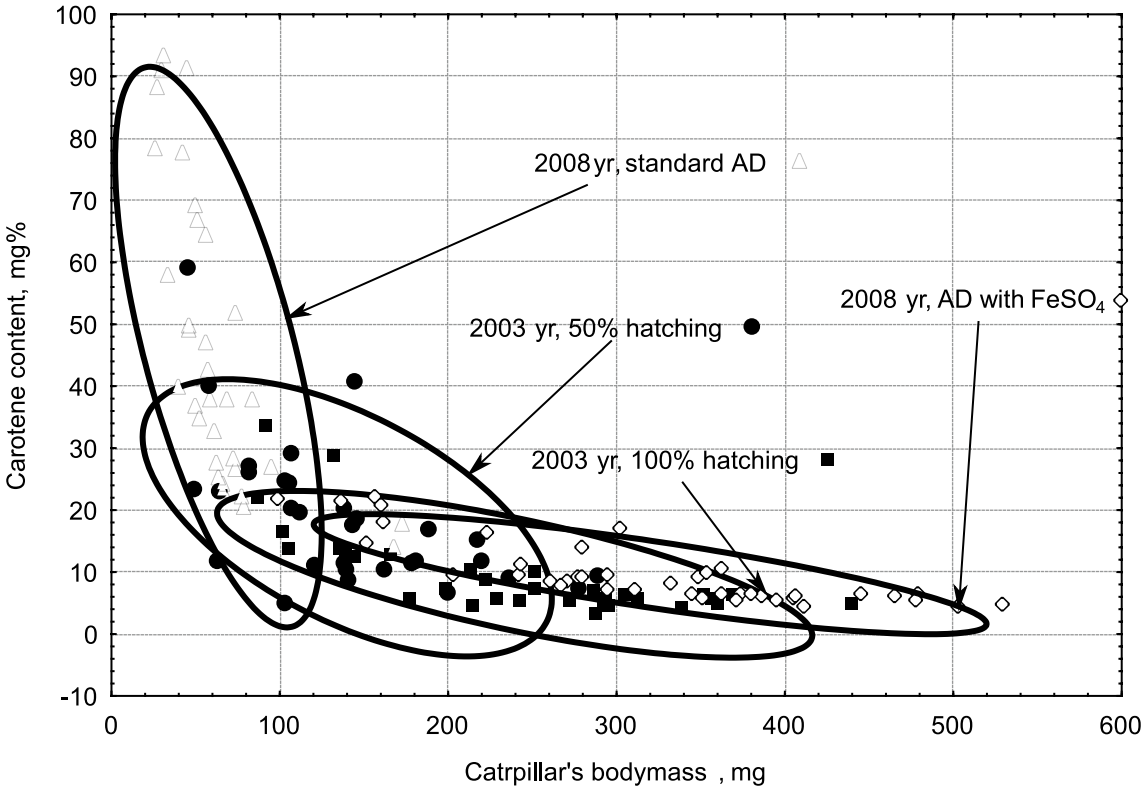


**Figure 1.** Regression analysis of dependence of carotene concentration on caterpillar body weight (measured at 5th instar)

ant on standard AD at 50% hatch and 2008 variant on standard AD.

Here we can observe a connection between diapause duration (shorter diapause in the northern populations of Gypsy moth is a well-known fact) and the activity of juvenile hormones and molt hormones (Lee et al., 1997), and also these hormones with free-radical activity (Alekseev, 2007). That is, it is possible that shorter diapause in the northern populations, among other acceptable reasons, is caused also by an orientation primarily to exogenous regulators of MLP. Much shorter diapause duration in 2008 (less than 2 months) than in 2003 (5 months) is indicative of such connection. Besides, trees of the same species but more adapted to low temperatures are known to have a high level of flavonoid contents, and in them the activity of MLP regulating enzymes changes (Sudachkova et al., 1997).

There are also data (Bogachyova, Zamshina, unpublished data) that Gypsy moth caterpillars at the latitude of Ekaterinburg, which is 80 kms north of the border area of outbreaks of mass reproduction, feed only on Rosaceae (apple tree, hawthorn). These species need higher iron contents in soil, and accumulate it in their fruits and possibly in leaves. Thus, feed selectivity of caterpillars is an indirect indication of higher orientation of Gypsy moth caterpillars to exogenous activators of MLP in the northern part of the area. We have caught Gypsy moth males in pheromone-bait traps in orchards at the latitude of Nizhniy Tagil (100 kms north of Ekaterinburg).

Besides, our data allow us to assume that one of the reasons of the expanded outbreak on the northern border in 1958-68 [defoliation range over 10 years (some years) amounted to more than 80 thousand ha] could be cold weather during caterpillar feeding. According to meteorological information, cool weather conditions (average monthly temperature in June did not exceed $16^0$C) preceded the outbreak of 1953-1954. From 1961 until 1964 the average monthly temperature in June was within limits of 15-$16^0$C and after that the outbreak area increased from 15,000 to 90,000 ha.

Thus, on the basis of our researches we can assume that the northward expansion the outbreak area of insect species is caused by individuals in populations switching over to exogenous regulators of MPL. Introduction into northern areas of some tree species

containing in their foliage (needles) high concentrations of these substances can be favourable for northward expansion of outbreaking insect species. Distinction in diapause duration between the northern and southern populations can be attributed not only to an adaptation to thermal spring provocations more often taking place in the south, but also to differences in the activity of endogenous regulators of MLP. Distinctions in the nutritive value of foliage (needles) of host species can depend on concentrations of nutrients and antifeedants, as well as on the concentration of exogenous regulators of MLP.

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# Mogući uticaj regulatora MLP u listovima biljaka domaćina na invazivnost filofagnih insekata

**REZIME**

Na severnoj granici područja rasprostranjenja gubara (*Lymantria dispar* L., 1758), gusenice se preorijentišu na egzogene regulatore peroksidacije lipida u membrani (MLP) u vezi sa povremenim periodima zahlađenja tokom ishrane. U slučaju uvođenja biljaka domaćina koje imaju povećane sadržaje egzogenih regulatora MLP (npr. $Fe^{2+}$) u listovima, koji mogu uticati na dužinu dijapauze, mogu se promeniti granice rasprostranjenja i intenzitet napada.

**Ključne reči:** Gubar; napad; regulatori peroksidacije lipida u membrani; dijapauza; infestacija