FACULTY OF FORESTRY

UNIVERSITY OF WEST HUNGARY

ANALYSIS OF GRADATION (2003-2006) OF THE GYPSY MOTH (*LYMANTRIA DISPAR* L.) AND NUTRITION INVESTIGATIONS ON THE GYPSY MOTH AND NUN MOTH (*LYMANTRIA MONACHA* L.)

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Markóné Nagy, Krisztina

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Gyula Roth Doctoral School of Forestry and Wildlife

Management Sciences

Head of the Doctoral School:

Prof. Dr. Faragó, Sándor

Ecology and diversity of forest ecosystem program (E1)

Head of Program:

Prof. Dr. Mátyás, Csaba

Supervisor:

Prof. Dr. Varga, Szabolcs

1. THE SIGNIFICANCE OF THE TOPIC

The gradation of gypsy moth (*Lymantria dispar* Linnaeus) effected exceptionally large areas in Hungary in the last decade. Its reason is that int he Transdanubian and northern part of the country the percentage of forested areas are high. Huge number of populations can be developed and in forests serving as main food sources, its caterpillars may cause serious damage or even economic loss. In Hungary oak trees are the most important tree species of forest management. Predominantly the Pedunculate oak (*Quercus robur*) and the Turkey (*Quercus cerris*) oak have the most important tree species are the main host plants of the gypsy moth, but it can cause complete mastication in most deciduous trees. Its periodic gradation strengthen those theories that mostly climatic factors, the presence of natural predators, the presence of host plants and their characteristics influence the formation of gradation. The dry years and uneven distribution of rainfall are disadvantageous for the health condition of forests. Appearance of certain pests and pathogens can be explained with this weakened condition. Periodic gradation of gypsy moth can characteristically be observed in years after long dry periods, that may occur in every 8-10 years depending on the weather. The dry period was the main reason that in 2003 the density of the gypsy moth population increased so much that they caused economic loss in forested areas and they also spread in agricultural fields, public areas and parks. The size of the damaged area had become the greatest ever, it exceeded more than 200 000 hectares by 2006. Chemical plant protection became necessary also in forested areas in this time. Determination of the area to be protected happened on the examination of several aspects; where among possibilities of the forecast the viability of egg masses, their cold tolerance and the migration of caterpillars had to be considered. It was necessary to examine where significant damage can be caused by the chewing of gypsy moth in the next year, which forest parts might be affected by total destruction. For this we have to know the factors having role in the selection of food source of the caterpillars, the metabolic processes going on in the host plant and the insect-host plant interactions. The knowledge of correlations makes the decision on chemical protection methods easier and the interests of environmental protection can also be enforced.

2. OBJECTIVES

The author's objectives were to analyse the biological features of the mass gradation of the pest gypsy moth lasting between 2003 and 2006 and the examination of phases of mass gradation. During this gradation having been bigger than ever before in Hungary several such biological features could be observed that had not been known in details, and new questions turned up about the pest's features already known. During the process of mass gradation the latency, the introductory phase, the outbreak, the collapsing, the ending and latency again have different characteristics, and these steps in case of huge populations can be characterized with more interesting correlations, and they have more interesting features as well. The analysis of biological features happened by the examination of the viability of egg masses.

In order to study the stages of mass gradation the correlations among the main forest constituting species, the location of forests, the condition of populations and their viability were analysed by the author. The cold tolerance of eggs was examined with an experimental series to demonstrate the effect of the extremely cold weather occurred in winter of 2005 on the process of gradation.

The species composition of natural predators and the rate of parasitism were examined in two consecutive years in pupae and in one year in eggs.

During the observation of feeding nature of caterpillars, it was supposed that for the impact of strong damage caused such chemical processes are taken place in the host plants that react upon the insect feeding on them. For this reason foliage of two Turkey oak stands were compared in two forests. There was a significant difference between the two stands, namely one of them was totally destroyed by the gypsy moth during the spring of the year of the experiment, while the other was not damaged.

In the next step, the author searched the literature for gaining experiences and examined that secondary metabolites playing the most important role in host plant-insect interactions, namely phenols and their types being present in Turkey oak in larger quantity how influence the caterpillars of the gypsy moth. These studies excluded any other influencing

factors. It was analysed how the indicators of development change in case of each phenol type.

Finally, another *Lymantria* species, the nun moth having smaller importance in Hungary but also causing serious damages in European countries was compared to the gypsy moth including its development characteristics. The aim of this study was to determine the morphologic and development characteristics of the two species excluding any influencing factor, on artificial nutrient substrate. With such type comparison of features of species feeding on different host plants her aim was to support the risk analysis if any of the above species appear.

3. RESEARCH METHODOLOGY

3.1. Viability analysis

Five forest parts in Veszprém County were designated for the study. The strength of hatching was determined with the examination of 1-1 egg masses collected from randomly selected 20 trees in each forest part. After taking them into laboratory, the 100 pieces of egg masses were put in Petri dishes one by one. Room temperature and natural light and dark periods were ensured for the hatching. The egg masses and number of hatching larvae were examined in every two days.

3.2. Examination of frost resistance of the egg masses of gypsy moth

The examination of frost resistance of egg masses happened in two periods: at the end of 2004, then in the spring of 2006.

At the end of 2004 the cold treatments last for 2, 5, 7 and 10 days. The temperatures of treatments were -10 °C and -20 °C. The treatments happened in a fridge with controlled temperature. The relative humidity was 70 % in the fridge. The examination of egg masses happened at room temperature (20-23 °C). On the days of the examination hatching larvae were counted and removed.

In March 2006 larvae still being in eggs, just before hatching thus having only a little amount of nutrients were examined after a 4-hour treatment at -15 °C and a 5-day treatment at -6,7 °C.

When the cold treatment ended at both groups, the sample and control Petri dishes were placed at room temperature. Then the number of hatching larvae was counted in every two days.

3.3. Examination of parasitism of gypsy moth eggs and larvae

Parasitism of pupae was examined in 2004 and 2005, while parasitoids living in egg masses were studied in the spring of 2005. Pupae parasitoids were grown in an insectarium, egg parasitoids were raised in Petri dishes then they were sent into the Insect Parasitological Laboratory of the Plant- and Soil Protection Service of Vas County for species determination.

3.4. Change of phenol concentration in the host plant occurring for the effect of damage caused by gypsy moth.

The aim of the study was the verification of change in internal composition of Turkey oak leaves due to the stress caused by the chewing of gypsy moth larvae with chemical analysis. The chemical change could be interpreted by the comparison of total phenol content of the foliages. The collection of samples happened during August in Veszprém County, in the surrounding of the village Márkó in two forest parts having similar habitat characteristics. In one of the forests the gypsy moth caused complete destruction in 2005, and the newly burst leaves were sampled. In the other area the damage caused by the gypsy moth was insignificant, thus leaves of non-damaged trees were sampled. All leaves were originated from Turkey oak. The analytical examination focusing on the determination of total phenol content was done by Department of Chemistry of Gyula Juhász Faculty of Teachers' College of the University of Szeged. For the determination of total phenol content the modified Price – Buttler method offered by H.D. Graham was applied. At the average of calibration curves the $y=52,45x – 0,0428$ equation was used.

3.5. Food source preference of gypsy moth as a function of phenol content

The author, in order to avoid influencing factors being present in nature examined the development of gypsy moth caterpillars beside controlled temperature and lightening on a semi-synthetic medium, in a laboratory. Beside the control, the medium was enriched with tannin, gallic acid and ellagic acid that occur in the highest quantity in the leaves, in the bark and in the acorn of Turkey oak. Two concentrations of each tannin were applied in the experiment. Raising of gypsy moth was done such way, that each caterpillar was placed in a separate Petri dish at room temperature. One complete generation was raised while on each third day the following parameters were measured:

- mortality,
- growing of weight from L_3 stage,
- date of entering into pupa state,
- weight of pupae.

3.6. The examination of the development of nun moth, and the comparison of the development of gypsy moth and nun moth

Similarly to the raising *Lymantria dispar* – excluding factors being present in natural conditions – the biological features of the nun moth were examined. The examination was performed in the Laboratory of BOKU Institute of the University of Vienna. For raising nun moth larvae a medium specially developed for *Lymantria monacha* was applied, which composition was originally developed by the US Forestry Service (USDA). The growth rate of weight, the maximum weight of pupae and the utilisation of nutrients were measured, and the differences between the development of male and female larvae were analysed on the basis of the above results.

Finally, results of larvae raised on normal medium during the study performed on the gypsy moth in 2009 were compared to the results gained by the experiment on the nun moth done in 2010.

4. RESULTS

Analysis of viability and cold tolerance was done on egg masses. The viability, and within this the number of larvae raised from egg masses in the last year of gradation was lower in case of those samples which were originated from the centres of gradation (infection).

The examination of cold tolerance showed that the low temperature has negative impact on wintering of egg masses, if the weather is cold in the first third of wintering and lasts durably. In opposite case, namely in the last month of wintering, temperature below zero does not cause significant egg mortality. These statements of the thesis support the proper judgement of the state of gradation and the effect of abiotic factors.

During the study of parasitism of gypsy moth pupae it was stated that in year 2004 the rate of total parasitism was lower than that of the samples taken a year later. The sample sites differed not only in species raised but in the number of parasitoids. In the examined two years the determinant species were the *Brachymeria intermedia* syn.: *Brachymeria tiabilis* and the *Theronia atalantae*. From the eggs the *Ooencyrtus kuvanae* and the *Anastatus japonicus* species had been developed.

Beside the abiotic effects and the natural enemies of the pest the author draws the attention of the effect of the host plant on the gypsy moth. It was proved that the plants react upon the chewing of gypsy moth larvae with producing secondary metabolites. Phenols produced such way may cause distorted development and high mortality of larvae, thus the fecundity of the population gets worse.

It was stated during the investigation of nutrition, diets, which contained ellagic acid or the tannin in 0,5% concentration had the most negative effect on larval development.

In the study of the development of the nun moth on artificial medium, the medium used first was appropriate from any point of view. Well known differences between the species could also be seen by excluding influences being present in natural conditions. By the results gained with the analysis of stages of development, vigour of growing and the utilisation of nutrients a risk analysis can be performed both regarding the gypsy- and the nun moths threatening with danger in Europe and also in overseas.

5. THESIS

5.1. In order to forecast of swarming of the gypsy moth, the present work examined the gradation and the damage of gypsy moth showed that adults that developed from wandering larvae, laid more eggs on new host plants. The number of larvae hatching from one egg-mass pointed at the number of viable embryo or larvae on the last year of gradation this depends on the host plant of laying egg generation; their physiological state and if they suffered by the gypsy moth or other herbivore insect. The author stated that natural enemies multiplied around the surrounding of egg-masses.

5.2. The dissertation is engaged in the analysis of cold effects on eggs in different periods of wintering. The author examined the resistance of egg-masses against cold temperature in several different time periods. It was shown that higher portion of the embryos died in the first period of wintering when eggs were placed at -20 and -10 °C temperature in a freezer for several days. With increasing the duration of cold period, the number of hatched larvae decreased in the examined time unit. In the last third of wintering not only the temperature below zero lasting for several days but also the several hour cold effect had no significant influence on the hatching of larvae, only it began a bit later than that of the control eggs. The results show if the low temperature occurs in the first part of wintering the viability of eggs or the size of population decreases depending on the duration of the cold effect.

5.3. In the investigation of parasitism of the gypsy moth pupae in 2004 and 2005 determinative parasitoid species were *Brachymeria intermedia* syn.: *Brachymeria tibialis* és a *Theronia atalantae.* From eggs *Ooencyrtus kuvanae* and *Anastatus japonicus* species developed.

5.4. The author proved that for the impact of the chewing of the pest such chemical reactions induced in the host plant that they influence the feeding habit of herbivore insects. The author has verified that:

- in the *Quercus cerris* the chewing habit of the larva of the gypsy moth causes biological change leading to the production of secondary metabolites, within this the measurable quantities of phenol increased.
- in identical environmental conditions, in the damaged foliage of the *Quercus cerris*, the phenol content of the leaves was nearly doubled, compared to undamaged foliage.

An objective of a further research can be the determination how long the changed chemical quality of the host plant endures and which factors may affect this apart from the chewing of herbivore insects.

5.5. The author compared the effect of three phenol types of *Quercus cerris* on the gypsy moth larval development with an artificial diet containing gallic acid, ellagic acid as well as tannin in two concentrations. The purpose of this experiment was to model the natural process of feeding development of larvae and to analyse the effect of various phenol compounds on the development of the gypsy moth. According to the results, even small phenol concentration caused small development-intensity and greater mortality. The ellagic acid and tannin have the most negative effect on larvae. As an apparent contradiction, using higher concentration (1%) of phenol types, larvae apparently adapted to the higher phenol concentration diet. The adaptation manifested in increased growing and weight and also in reduced mortality of larvae compared to individuals feeding on diet with low phenol concentration. The results of the fecundity examination showed that the diet containing tannin, differently from gallic acid and ellagic acid, resulted in viable adults but their fecundity was significantly worse than that of the control larvae.

5.6. During the examination of the development of the nun moth and the gypsy moth, firstly a specific artificial diet developed for the nun moth was tested. In this study the author was the first who used this method effectively. During raising the larvae - excluding factors being present in the nature - development indices were set for both species. Her results may support the pest risk analysis (PRA) of these insects in European risk zones, the correct decision for plant protection and the development of action plans.

6. THE AUTHOR'S PUBLICATIONS IN THIS TOPIC

6.1. Publications:

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