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# ECOPHYSIOLOGICAL METHOD FOR ASSESSMENT OF *ORCHESTES FAGI* L. INFESTATION ON COMMON BEECH TREES

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## Abstract

The paper presents an applicable method for investigation and assessment of the damage caused by beech weevil (*Orchestes fagi* L.) on the leaves of Common beech (*Fagus sylvatica* L.). Selecting of appropriate ecophysiological approaches is a workable tool for an assessment of leafminers' effects as these methods can be applied for leaves with a gradient of damaged area. Thus, it can be established a tendency in the attack effect and determined the limits of the plant protective system. This methodology was applied successfully and can provide reliable set of data for clarifying the relationship between the mining larvae, developing in the infested leaves, and the host plant. The application of this methodology in other environmental conditions and woody species could contribute to a development of biological means for a control on the leafminers in the forests.

**Key words:** ecophysiology, leafminer, plant defense system, process disruption.

## Introduction

Leafminers are a serious problem for the most of tree species in Bulgaria. The complex of leafminers may cause up to 78 % of the total damaged area of beech leaves (Dimitrova-Mateva 2008). Our previous investigations have established that *Orchestes fagi* L. is the first in importance in the group of leafminers on Common beech leaves whose feeding induces alterations in the activity of some antioxidant systems (Miteva et al. 2007, Dimitrova-Mateva et al. 2014). The growth of beech weevil in our country is connected with the Common beech (*Fagus sylvatica* L.) and Oriental beech (*Fagus orientalis* Lipsky) trees. Damages are observed both by the adults and also by the larvae of *O. fagi*. During its feeding the imago makes holes on the beech leaves. This allows an estimation to be made for a degree of infestation by beech weevil. The imago destroys an average of about 20 % (in the particular cases up to 93.2 %) of the photosynthetic leaf area (Dimitrova-Mateva 2008).

Feeding of the larvae is tightly connected with the parenchyma layers of the leaves. Larvae make a large rounded mine which is visible from both leaf sides. It initiates from the midrib and goes to the top of leaf blade, whereas two epidermal layers remained unaffected. The accurate assessment of the defoliation is difficult to be made as an absence of a visible loss of leaf area.

In the mined leaves arises physiological stress, provoked by the interaction between insect and host plant. On one side, the insect damages leaf tissue and induces plant's response. On the other, the physiological activity of larvae affects the physiological and biochemical processes of the host plant (Dimitrova-Mateva 2014). This imposes the necessity a new approach to be applied for an assessment of infestation effect by leafminers.

In Bulgaria so far has been applied mainly a morphological analysis of damaged beech leaves. Examination of artificial defoliation of *F. orientalis* seedlings established that the reduction of the photosynthetic area leads to decrease in the annual growth (Stalev 1990, 1991). The author has tried to estimate the defoliation by leaf chewing insects, considerably different from that, caused by leafminers.

The first steps in the biochemical analysis of infested beech leaves have showed that the feeding and developing of the beech weevil larvae causes remarkable changes in the enzymatic and non-enzymatic components of plant defence system as endogenous phenols, low molecular thiol compounds and products of lipid peroxidation (Miteva et al. 2007).

In Europe, where is the natural region of *Fagus sylvatica* L., such kind of researches have not been made. There are no systematic investigations on the changes in the physiological activity of any forest tree species caused by infestation of leaf-mining insects. Estimation has been made on the germination of *Aesculus hippocastanum* L. at a strong mining of leaves of *Cameraria ohridella* Deschka & Dimic (Takos et al. 2008). Isolated analyses of photosynthesis are made on mined leaves of some grasses, fruit trees and agriculture species (Hespenheide 1991).

Separate studies established, that as a result of damages, caused by *Orchestes fagi* L., the vitality of *Fagus grandifolia* Ehrh in North America weakened and trees become more susceptible to the root fungi from genus *Armillaria* sp. (Sweeney et al. 2012). It was observed a preliminary fall of beech leaves (Pritchard and James 1984), which may be regarded as a mechanical defence (Dimitrova-Mateva 2014).

The larvae start to develop still in the beech buds and cause reduction in the infested leaf area. At a weak level of infestation the physiological processes in the unaffected leaf area can be stimulated and do not show a negative effects on the plant (Retuerto et al. 2004). Due to the faster flow of assimilates in some kind of damages the rate of photosynthesis increases and thus compensates the reduction in the leaf area (Meyer and Whitlow 1992). Stimulating effect of the attack by other insects on the physiological activity was observed even in the lightly infested leaves. In very strong infestation, when most of the leaf area is affected, the physiological indicators decreased sharply (Anev et al. 2013). The preliminary drying and falling of the leaves causes not only a lack of assimilates during the growth period but also decreases plants' ability to accumulate nutrients for the unfavourable winter period (Eyles et al. 2011). This serves as a predisposition for the weakening of plant and its defensive system in the winter as well as for a delayed and weakened start in the next growth period and finally leads to gradually exhausting of the whole plant.

This paper presents a set of ecophysiological methods for an investigation of beech weevil *Orchestes fagi* L. on the leaves of Common beech. The complex methodology consisted of four groups of methods and was applied successfully in young beech forests at two altitudes in the region of Western Balkan mountain (Anev et al. 2015, Chaneva et al. 2015a, Chaneva et al. 2015b, Dimitrova-Mateva et al. 2015).

## Morphological Analysis of Beech Leaves

Leaf material was collected during the entire growth period in samples plots at two different altitudes (Anev et al. 2015). In order to be determined the level of infestation by beech weevil randomly chosen leaves from the lower crown parts of stock trees were analyzed (Stalev 1990, 1991; Nielsen and Ejlersen 1977; Nielsen 1968, 1978). Biometric parameters of the leaves (total leaf area, damaged and undamaged area, total leaf length and width as well as a length of petiole) were determined with open source software ImageJ (Rasband 2016).

The established leaf damages were classified in the following groups: number of mines and mined area by *O. fagi*, chewed area by the adults of beech weevil, mined area and number of mines, caused by leaf mining moth *Ph. maestingella*, damages by gall-makers and leaf-chewing insects.

The data collected allow to be calculated:

**1. Frequency** of the every one of the insect species or group of pests as a percentage of the infested leaves to the total number of the analyzed leaves (Connor et al. 1983, Vorontzov et al. 1991).

**2. Participation of each type of damage** as a percentage of the total leaf area and the total area of damage.

**3. Coefficient of the quantitative density (*K*)** – the number of the individuals per leaf area (Nielsen and Ejlersen 1977, Nielsen 1978):

$$K = \frac{Z}{N}.p.m.t$$ (according to Segebade and Schaefer 1979)

where:

*N* – number of observed trees;

*Z* – number of infested trees;

*p* – ratio between the number of infested leaves and the total number of leaves;

*m* – an average number of mines to the number of the holes on a leaf;

*t* – percentage of damaged area to the total leaf area.

**4. Index of the degree of damage (*R*)** (Rosnev et al. 2007)

$$R = \frac{\sum n.k.100}{N.K}, \%$$

where:

*n* – number of the model trees with a definite degree of infestation;

*k* – degree of infestation;

*N* – total number of model trees;

*K* – maximal degree of infestation.

This formula is applied in the field of forest protection for defoliation assessment of whole trees (Rossnev et al. 2007), using five degrees of infestation (*k*) by the following percentages of defoliation: 0 (0–10 %), 1 (11–25 %), 2 (26–59 %), 3 (60–99 %) и 4 (100 %). Previous studies have shown that the damage from leaf insects rarely exceeds 30 % (Dimitrova 2005, 2008). For this reason different type of grouping was determined in dependence on the highest recorded level of attack. Such exemplary grouping (Table 1) was used in our field experiments in order to determine the relationship between the level of infestation and the physiological and biochemical parameters (Anev et al. 2015).

**Table 1. Degree of infestation (k) of the leaves depending on their damage.**

| Degree of infestation | Group |
|---|---|
| 0 – 20 % | 1 |
| 20 – 25 % | 2 |
| 25 – 30 % | 3 |
| > 30 % | 4 |

### Gas Exchange and Chlorophyll Content of Beech Leaves

Measurements of the rate of basic physiological indicators (photosynthesis, transpiration, and stomatal conductance) during the growth period were performed by using Portable Photosynthetic System Li-6400 for an infrared gas analysis (Li-Cor 2012) on attached beech leaves with different degree of infestation. The total content of chlorophyll was determined on living leaves with a portable chlorophyll meter AT LEAF by the method of non-invasive measurements on the basis of difference in the optical density between 660 nm и 940 nm.

### Biochemical Analyses of Beech Leaves

After a determination of the level of damage, rate of gas exchange and total content of chlorophyll the sampled leaves were fixed in liquid nitrogen for subsequent biochemical analyses.

The concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids were measured spectrophotometrically after an acetone extraction (Arnon 1949).

Malonedialdehyde (MDA) content was measured according to Dhindsa et al. (1981). Endogenous hydrogen peroxide ($H_2O_2$) was determined spectrophotometrically at 390 nm (Jessup et al. 1994). The determination of free proline levels was done according to Bates et al. (1973). The content of soluble proteins was measured by method with Kumasi Brilliant Blue G250 (Bradford 1976). The activity of enzymatic defence system was determined by methods as followed: superoxide dismutase (EC 1.15.1.1) – after Beauchamp and Fridovich (1971); guacol peroxidase (EC 1.11.1.7) – after Hart et al. (1971); catalase (EC 1.11.1.6) – after Aebi (1984).

The histochemical detection of active oxygen ions was made with the method of Sakamoto et al. (2005). The content of hydrogen peroxide and superoxide anions – with the method of Shinogi et al. (2003). The content of phenols was determined by the method of Singleton et al. (1999).

### Statistical Analysis

All measurements were processed statistically in the following order: tests for a type of data distribution, descriptive statistics and ANOVA-test for estimation of the significance of the differences among the groups of infestation in relation to the investigated physiological and biochemical parameters on the respective altitude.

## Conclusions

The presented methodology is an original complex of classic and up-to-date ecological observations, physiological measurements and biochemical analyses of the infested by the beech weevil Common beech leaves. This methodology was applied successfully and can provide reliable set of data for clarifying the relationship between the mining larvae, developing in the infested leaves, and the host plant. Its application in other environmental conditions, and other woody species, will be a contribution to the development of biological tools for leafmining pests control in the forests.

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