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# ADDITION TO ANALYSIS OF MORPHOLOGICAL PARAMETERS OF MINES ON TWO INVASIVE LEAF-MINING LEPIDOPTERA SPECIES (*PARECTOPA ROBINIELLA* CLEMENS, 1863 AND *PHYLLONORYCTER ROBINIELLA* CLEMENS, 1859) ON BLACK LOCUST

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The article presents results on analysis of morphological parameters of mines made by two invasive leaf blotch miner moths *Parectopa robiniella* (Clemens, 1863) and *Phyllonorycter robiniella* (Clemens, 1859) on the leaves of Robinia pseudoacacia in conditions of green zones within Dnipro city. It has been found that classical approach to measuring the mine areas is not sufficient for identification characteristics of feeding behavior of the studied insect species. A combined method on analysis of morphological parameters of leaf mines is proposed for measuring the caterpillars' feed activity, taking into account the volume of leaf tissue consumed.

*Forest leafminer (Gracillariidae Stainton, 1854), adventitious species, morphology of mines.*

According to modern concepts [10], biological invasion is considered as hazard to protection of natural biological diversity that is second in order of importance to destruction of natural habitats. Irreparable environmental damages can often result from consequences of this invasion [1, 3, 5, 6]; they can lead to significant biological disturbances in the being of entire ecosystems, resulting in massive economic losses in various industry sectors.

Currently, 187 Lepidoptera species are known to spread within Europe [7]. Therefore, 90 species are native to this part of the world. Invasion of these species is correlated with artificial expansion of the feeding plant area. The other 97 species are exotic and were accidentally introduced by humans into a new territory together with potentially suitable feed object. Most of exotic insect species came to Europe in the second half of the XX century. Of them, origin of 78 species was determined [7].

Nowadays, miners are spread widely within the adventitious forest insect species on the territory of Ukraine; effect of them increased among other phytophages because of high adaptive capacity to high level of contamination, moisture deficit, insecticide effects, and also due to the large amount of generations per year [2].

In Ukraine, history of of adventitious mining Lepidoptera invasion accounts for more than 25 years. During the period, four invasive species belonging to the leaf blotch miners family are of the greatest concern (Gracillariidae Stainton, 1854) – the horse chestnut leafminer (*Cameraria ohridella* Deschka et Dimić, 1986), the lime leafminer (*Phyllonorycter issikii* Kumata, 1963), the

locust digitate leafminer (*Parectopa robiniella* Clemens, 1863) and the locust leaf miner (*Phyllonorycter robiniella* Clemens, 1859).

The main indicator of invasive species stability in new environment is characteristic of its trophic relationships. Objective of our survey was determination of feeding activity of black locust (*Robinia pseudoacacia* L.) miners' invasive complex on the basis of morphometric parameters of their mines.

## Materials and Methods

The morphological studies of mines were carried out in green areas (parks, squares, and the Botanical garden of Oles Honchar Dnipro National University) in Dnipro city during the growing season of 2018.

In our study, the number of mines was calculated on the model branch. A model tree and a random branch on the tree with a length of at least 1 m was selected by randomization method. After that, the number of compound leaves and the number of mines on rakhis made by *Parectopa robiniella* and *Phyllonorycter robiniella* were recorded.

The mine-damaged leaves of *R. pseudoacacia* were photographed with a digital 5-megapixel camera using a binocular microscope MBS-10. Measurements were calibrated with a stage micrometer. Measurements were carried out using photos in the program ToupView 3.7. Length of mine (L) was measured parallel to the midvein, maximum width of mine (W) was measured perpendicularly to the line of its length; mine area (S) and leaf area (Sl) were also measured. The length:width ratio of mine (L/W) and the ratio of mine area to lamina area (S/Sl) were calculated (Fig. 1).

Statistical data processing was carried out by conventional methods using software packages for personal computers Microsoft Excel 2007 and STATISTICA 6.0.

## Results and Discussion

The main indicators of the miner caterpillars' feed success are considered to be morphological characteristics of mines. Assessment of mine area determines the number of feeding larvae, as well as their feeding activity. For estimation the distribution areas of two types of mines Fodor and O. Hâruța [4] scanned mine

on the leaves at 600 dpi (Scanner Mustek 1200 CP) and measured digitally. The samples were used to estimate mine areas and other morphometric measurements, such as shape index (area/perimeter) and elongation (long axis /short axis; if the value is close to 1, the object shape is close to a round). Shape index and elongation are shape descriptors, and they reflect differences in shape between the two types of mines. Shape index describes the contour complexity while elongation describes the deviation from round shape (which is about 1) [4].

To compare the results of data observed with the results of randomized test, E. Fodor and O. Hâruţa [4] also calculated the standardized effect size (SES). SES measures the number of standard deviations wherein the index observed (randomized) is above or below the average index of simulated data. The authors used standard deviation of 5000 simulated indices to calculate SES by the formula: SES = (Iobs–Isim) / Ssim. Assuming a normal distribution, SES values fall between –2 and 2 (below –2: indicates aggregation, between –2 and 2: random joint appearance and above 2: a separate appearance of the species).

In work of E. Fodor and O. Hâruţa [4], the authors used coincidence analysis to compare frequency of co-occurrence mines from two species on the same leaf (C-score) [8] based on data obtained from mines presence or absence. The C-score reflects degree of probability with which mines of both types occur on same leaf; it quantifies the number of mining units corresponding to each pair of mines [9]. For each species number of chessboard blocks (Ri-S) (Rj-S), where Ri is the number of occurrences mines of species I, Rj is the number of occurrences mines of species J, and S is the number of compound leaves samples [4]. C-score is the average number of mines for each pair of species. When the index is large compared to the zero distribution, segregation level will be less than expected. When the index is small, species aggregation is larger than expected.

According to our data (Table 1), average length of (*L*) *Parectopa robiniella* mine greater than the length of *Phyllonorycter robiniella* mine; wherein standard deviation of *Phyllonorycter robiniella* mines is greater, it indicates a large variability in mine length of the latter. Accordingly, coefficient of variation is lesser in *Parectopa robiniella*.

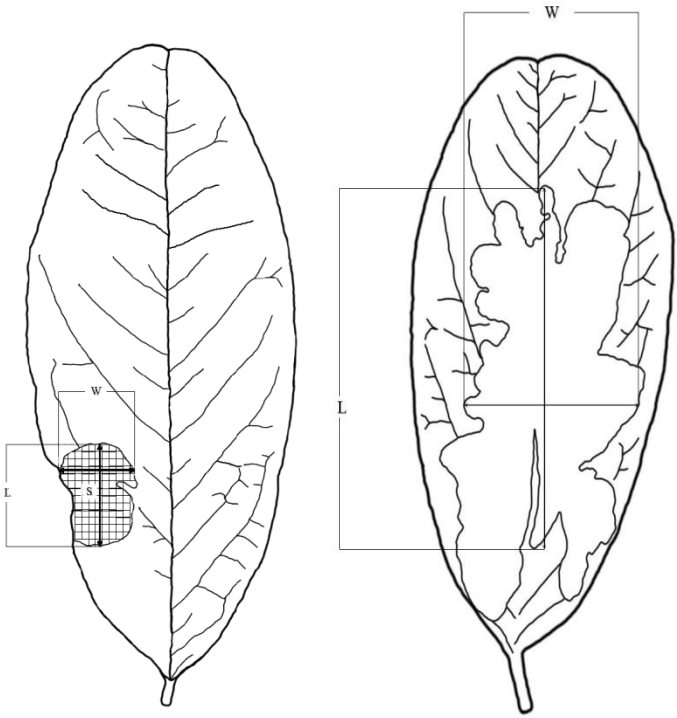


Figure 1– Schematic image of mines shape: a-Phyllonorycter robiniella, b – Parectopa robiniella; L – mine length, W – mine width, S – mine area

However, *Parectopa robiniella* has greater average width of mine (*W*) and average area of mine (*S*), while standard deviation in this parameter is greater than that of *Phyllonorycter robiniella*. Average leaf area (*Sleaf*), on which the studied species make mines, was greater in *Phyllonorycter robiniella*.

Table 1 – Morphometric variability of *Parectopa robiniella* and *Phyllonorycter robiniella* mines (n = 32) on leaves of black locust in the Park named after the 40th anniversary of the liberation of Dnipro

| Characteristics | *Parectopa robiniella* | | | *Phyllonorycter robiniella* | | |
|---|---|---|---|---|---|---|
| | SD | x | CV | SD | x | CV |
| L | 5.84 | 17.19 | 0.34 | 7.27 | 15.80 | 0.46 |
| W | 4.81 | 11.06 | 0.43 | 3.34 | 7.31 | 0.46 |
| S | 2773.61 | 5164.61 | 0.54 | 1770.01 | 3641.25 | 0.49 |
| Sleaf | 12420.07 | 33662.23 | 0.37 | 14691.42 | 34372.41 | 0.43 |
| L/W | 0.81 | 1.97 | 0.41 | 0.95 | 2.15 | 0.44 |
| S/Sleaf | 0.07 | 0.17 | 0.44 | 0.06 | 0.11 | 0.51 |

Note: CV – Coefficient of variation; SD – Standard deviation; *x* – Arithmetic mean; L – length of mine, W – width of mine, S – area of mine, Sl – leaf area, L/W – ratio length:width of mine, S/Sl – ratio area of mine: area of leaf lamina

The less elongated form of *Parectopa robiniella* mine leads to the fact that ratio length:width of mine (*L/W*) in *Parectopa robiniella* lesser than that of *Phyllonorycter robiniella* mines. Therefore, share of leaf surface occupied by the mine (*S/Sleaf*) is greater in *Parectopa robiniella*.

## Conclusions

1. The studies have shown that average length (*L*) of *Parectopa robiniella* mine greater than that of *Phyllonorycter robiniella* mine; wherein standard deviation of *Phyllonorycter robiniella* mines is greater, it indicates a large variability in mine length of the latter.

2. Average leaf area (*Sleaf*), on which the species studied make mines, was greater in *Phyllonorycter robiniella.* However, *Parectopa robiniella* has greater average width of mine (*W*) and average area of mine (*S*), while standard deviation in this parameter is greater than that of *Phyllonorycter robiniella*.

3. Analysis of the results showed that classical approach to the measurement of morphological parameters of mines (area, length and width) is not sufficient for determination feed activity of miner caterpillar.

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# ДО МЕТОДИКИ ВИЗНАЧЕННЯ МОРФОЛОГІЧНИХ ПАРАМЕТРІВ МІН ДВОХ ВИДІВ МІНЕРІВ-ІНВАЙДЕРІВ (*PARECTOPA ROBINIELLA* (CLEMENS, 1863) І *PHYLLONORYCTER ROBINIELLA* (CLEMENS, 1859)) РОБІНІЇ ПСЕВДОАКАЦІЇ

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Історія інвазії адвентивних видів лускокрилих-мінерів на території України нараховує понад 25 років. За цей період спостережень найбільше занепокоєння викликають чотири види-інвайдери, представники родини молей-строкаток (Gracillariidae Stainton, 1854), які на початку XXI ст. поширились всією територією Степової зони України. В статті наведено результати морфологічних досліджень мін, утворених двома інвазійними видами молей-строкаток *Parectopa robiniella* (Clemens, 1863) та *Phyllonorycter robiniella* (Clemens, 1859) на листках робінії псевдоакації (*Robinia pseudoacacia* Linnaeus, 1753) в умовах зелених зон м. Дніпро. Морфологічні характеристики мін можна сприймати у якості головних показників успішності живлення гусені мінерів. Оцінка площі міни визначає кількість личинок, що живляться, а також активність їх живлення. Морфологічну оцінку здійснювали шляхом сканування мін на листках (600 dpi) із подальшим цифровим вимірюванням. Відсканованні зразки використовувались для оцінки площі мін та морфометричних показників таких як індекс форми та подовження. З'ясовано, що індекси форми та видовження є дескрипторами форми, і вони відображають різницю по формі між двома видами мін. Індекс форми описує складність контура, у той час як подовження описує відхилення від круглої форми. Дослідження показали, що середня довжина міни *Parectopa robiniella* більша довжини міни *Phyllonorycter robiniella*, при цьому стандартне відхилення у мін *Phyllonorycter robiniella* більше, що свідчить про більшу варіабельність довжини міни в останніх. З'ясовано, що класичного підходу вимірювання площі мін не достатньо для визначення особливостей живлення досліджених видів. Для визначення активності живлення гусені запропоновано комбіновану методику визначення морфологічних параметрів мін із урахуванням об'єму вжитих тканин листка.