BIOLOGIJA. 2022. Vol. 68. No. 2. P. 101–109

Do some characters of leaf mines of Nepticulidae depend on anything else than the characeristics of the species itself?

Viktorija Dobrynina

State Research Institute
Nature Research Centre,
Akademijos g. 2,
Vilnius 08412, Lithuania

The article examines the aspects of dependence of some leaf mine characters. The correlation between the deposition of frass and the morphological type of the leaf mine suggests that ecological adaptations associated with the drying of the frass are diverse, depending on the morphological type the leaf mine. The colour of frass has little in common with the systematic group (i.e., family) of host plants. Black frass is characteristic and predominant among miners of any host plant family. The study also suggests that the location of frass is at least partially associated with different morphological types of leaf mines. When summarising the results of the study, it can be stated that the filling of the initial part of the mine with frass is related to the filling of the whole gallery of the leaf mine.

Keywords: leaf miners, Lepidoptera, pygmy moths, minology, plant miners

INTRODUCTION

The life mode of the larvae of pygmy moths (Nepticulidae) is distinctive, highly specialized, and endobiotic: the larvae live and feed within the green tissues of plants. Such ecological adaptation is called 'mining', and the damage to the green tissue of the plant (a cavity made by an insect larva which is covered by epidermis or at least cuticle), 'a mine' (for more information, see Diškus, Stonis, 2012). Leaf mines, the most important products of larval activity of Nepticulidae, in many cases can be used for species identification. Mines vary in their location on the host plant; they are also different in their morphological type, winding, and filling of the mine with frass, including frass colour (see Stonis et al., 2022). It should be mentioned that the deposition of frass in a mine is not only a significant diagnostic character but also appears to be an important ecological adaptation. In order to prevent the development and spread of dangerous fungi or bacteria, larvae living in a closed cavity have to dry the frass. Different species of Nepticulidae have adapted to doing it in a variety of ways: by leaving frass in a narrow or wide line, arranging it in an interrupted line (dotted line) or depositing spiral (coiled) frass, etc.

There have been papers published that deal with the morphology of leaf mines. For example, some attempts have been made to analyse leaf mines by using digital video tools (Dai et al., 2011,

* Corresponding author. Email: viktorija.dobrynina@gmail.com

2012). In these papers, some parameters of serpentine (linear) leaf mines and their shape were examined, including mean of the turn angle, concentration of turn angles, fractal dimension, straightness, sinuosity (winding), etc.

It should be noted that Lithuanian researchers have also made their contribution to minology. A couple of significant monographs on the diversity of the Lithuanian Nepticulidae, their leaf mines, and trophic relationships were published one or two decades ago. One of them is the monograph 'The Nepticuloidea & Tischerioidea (Lepidoptera), – a global review, with strategic regional revisions' by Puplesis and Diškus (2003). Another, a no less valuable book, is 'The Nepticulidae (Lepidoptera): taxonomy, chorological composition and trophic relationships' by Diškus and Stonis (2012). In this highly illustrated publication, the mines are presented as belonging to different morphological groups and types, as well as some other ecological data are provided (including original photographs of leaf mines of the Lithuanian Nepticulidae (Diškus, Stonis, 2012).

In the most recent paper, various diagnostic characters of leaf mines are identified, grouped, and illustrated, with the calculation of their detection frequencies (Stonis et al., 2022a). It is also very important to mention the most recent publication by Lithuanian researchers 'A guide to leaf mines of the Lithuanian Nepticulidae' by Stonis et al. (2022b). It is the first major diagnostics guide for Lithuania, which, among other things, provides a schematic breakdown of morphological and diagnostic characters of leaf mines (Stonis et al., 2022b).

The purpose of the current article is to find out if some characters are in any way connected to other characters of the same leaf mines. For this purpose, mines of all species of the Lithuanian Nepticulidae were examined. The task was to determine whether the frass in the initial part of the leaf mine is related to the deposition of frass in the main part of the leaf mine and to find out if there is a dependence between frass and the morphological type and whether there is any correlation between the colour of frass and the host-plant family.

MATERIALS AND METHODS

The study sample, which was based solely on the leaf mines of the Lithuanian Nepticulidae, comprised, 77 species in total. The studied Nepticulidae belong to the fauna of the temperate forest biome (Stonis et al., 2022a). Following the pictorial scheme of the morphological characters of Nepticulidae leaf mines by Stonis et al. (2022a), a character-coding datasheet was compiled for leaf mines of each species of the sample. In this datasheet, morphological characters of leaf mines were marked (coded) following Stonis et al. (2022). Although most studied taxa of Nepticulidae were characterised by a single character in each group of characters, there were species in which two or three characters were found in the same character group. In the latter case, all detected characters were marked in the datasheet and counted later in our analysis regardless of their frequency in the sample.

Methods of leaf mine collecting were provided and illustrated by Diškus, Stonis (2012). Numerous leaf mines of the studied sample of the Lithuanian Nepticulidae were recently documented and published by Stonis et al. (2022b).

RESULTS

Filling of the initial part of the leaf mine with frass. The filling of the mine with frass is both an important diagnostic character and an ecological feature of Nepticulidae. The larvae of Nepticulidae can start mining either by completely filling the gallery with frass or by forming a central line of frass. The initial part of the mine may also, but very rarely, occur in a petiole or other part of the plant. Such cases were not analysed in this paper.

We found that larvae of most of the species completely filled the initial part of the mine with frass (48 species), and some species have the central line of frass and unfilled clear margins of the gallery (44 species). It is noteworthy that the numbers of species overlap: we found that some species could have both characters or intermediate states of the characters.

The purpose of the study was to determine whether the deposition of frass in the initial part of the mine was related to the deposition of frass in the main part of the mine. The larvae of Nepticulidae are variously adapted to fill the main part of the mine. The filling of a mine with frass can have the following characteristics: (1) a slender line of frass but wide unfilled (clear) margins of the gallery, (2) a fairly wide line of frass but wide unfilled margins, (3) a wide band and slender clear margins, (4) the entire width of the gallery filled with frass, and (5) a mine without the frass line or band (but accumulated in a blotch or clumps).

The study found out that mines with a central line of frass in the initial part of the mine usually were characterised by either a slender frass line and wide unfilled (clear) margins in the main part of the mine or by a relatively wide frass line with wide clear margins (27 species in each group). Mines with their initial part completely filled with frass were characterised by either an absence of a line (band) of frass or by a specific arrangement of frass when the entire width of the main gallery of the mine was filled with frass (24 species) (Fig. 1).

The data also showed that when the initial part of the mine was filled with frass, often the main gallery had a wide line or band of frass and narrow unfilled margins (24 species) (Figs 1, 2).

Dependence of frass deposition on the morphological type of the leaf mine. As regards the deposition of frass in leaf mines, the Nepticulidae species are not homogeneous. In the study, most species were identified as forming a continuous line of frass (56 species). Other species possess/have the following characters: a dotted deposition of frass (dotted line) (21 species); a spiral frass line (26 species); with frass accumulated in a large, rounded blotch or oblong clumps (22 species); and irregularly scattered frass (27 species).

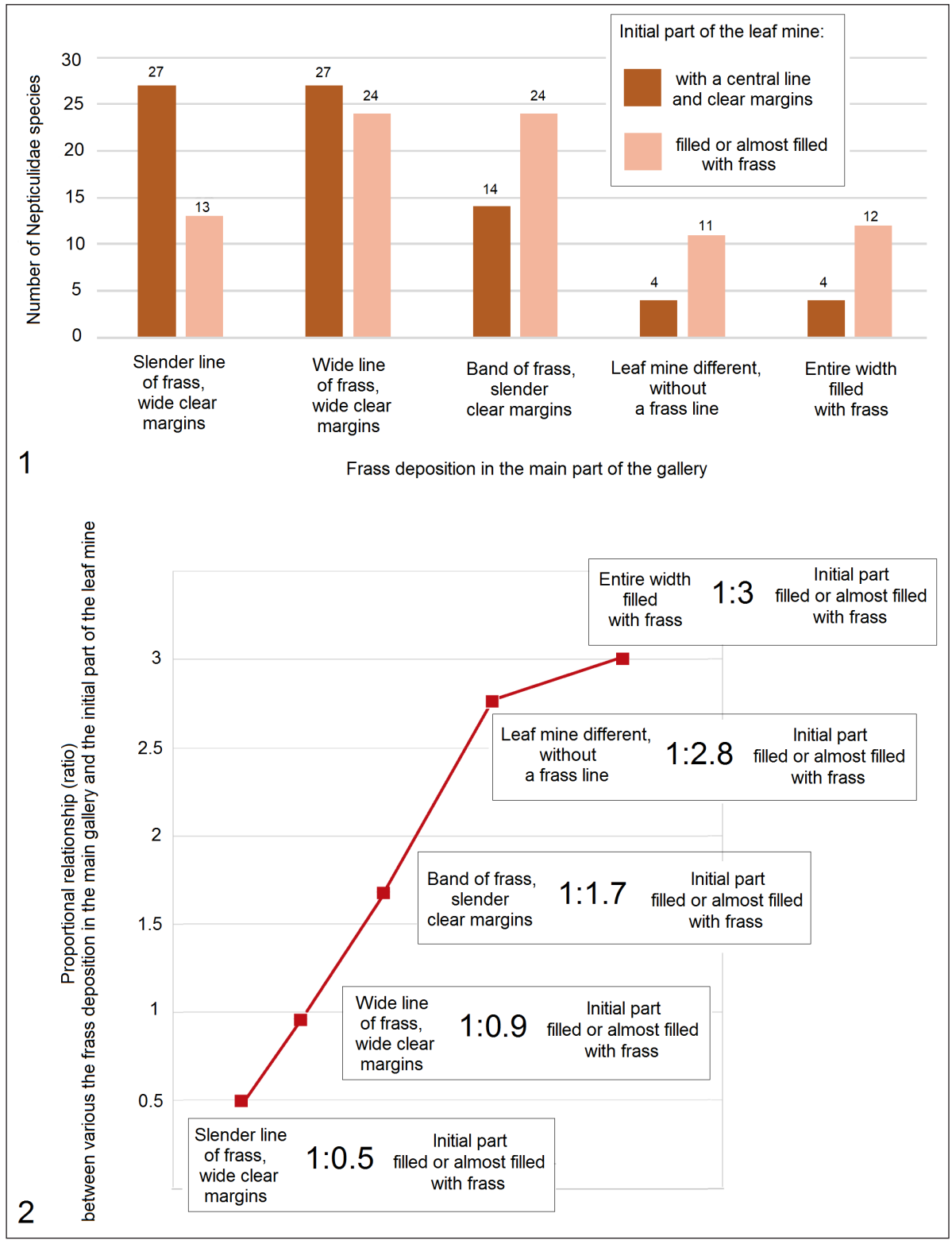
For each studied species, the morphological type of the leaf mine was assigned: spiral, slender serpentine (i.e., serpentine, gradually slightly widening), expanding serpentine (i.e., serpentine, strongly widening), false blotch, combined, and blotch-like.

The analysis showed that the Nepticulidae species which deposit their frass in a continuous band were most often associated with the morphological type of slender serpentine mines; 32 species (or 38%) were classified as such. Among the species with a continuous line of frass, the expanding serpentine leaf mines account for 30% (25 species) and false blotch ones for 14% (Fig. 3). All other types of frass deposition in relation with the morphological type of the leaf mine are shown in Figs 4–7. Most of the species that deposit frass in a dotted line belong to the morphological type of expanding serpentine mines (14 species or 45%).

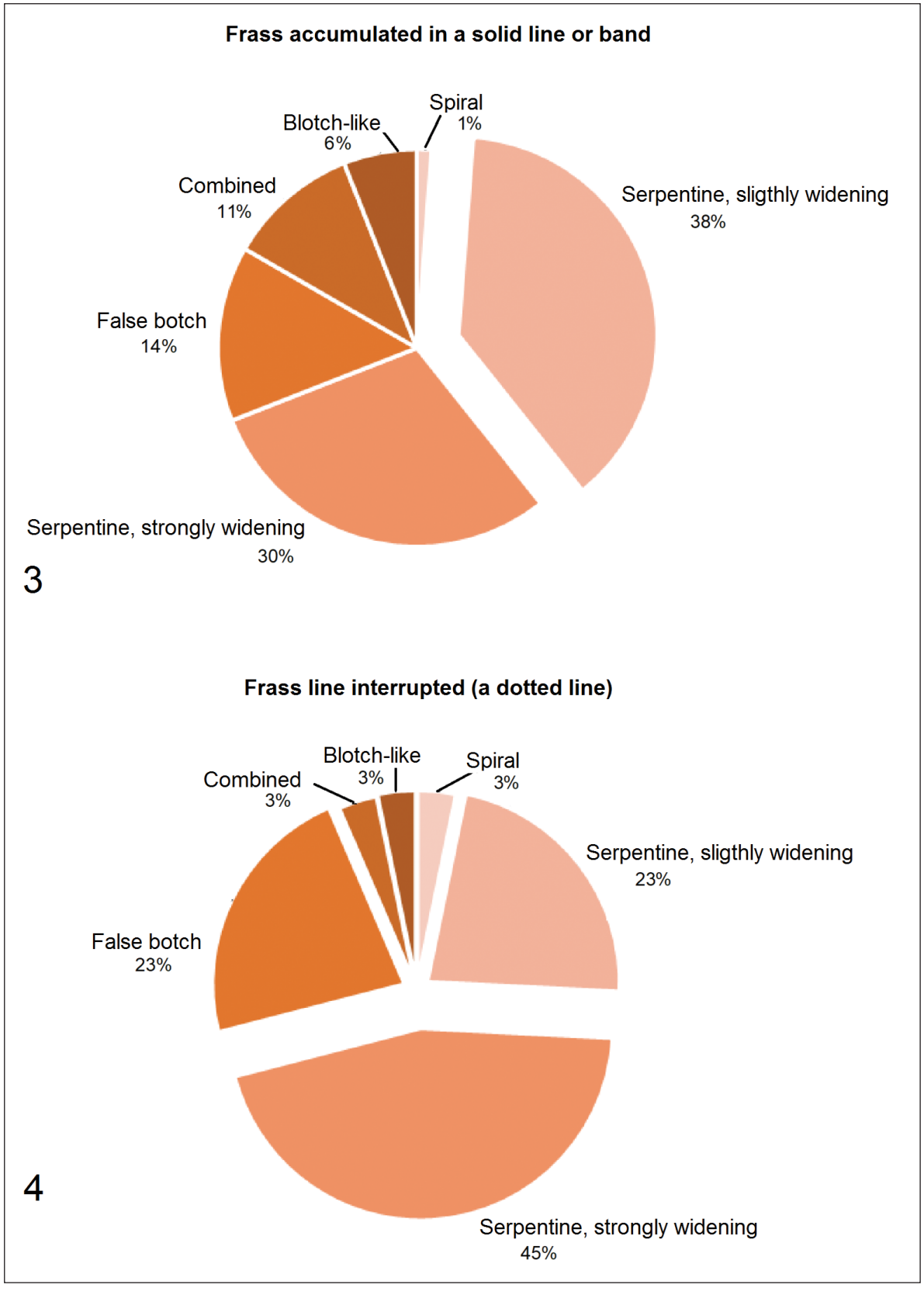
The data also showed that frass accumulated in a large blotch or oblong clumps generally occurred in leaf mines of the following two – combined or blotch-like – morphological types, both of them comprising the same number of species, 11 species or 29% each. Irregularly scattered frass is most common in the following morphological types: expanding serpentine leaf mines accounting for 32% (15 species), false blotch leaf mines accounting for 25% (12 species), combined leaf mines accounting for 17% (8 species), blotch-like leaf mines accounting for 15% (7 species), and slender serpentine leaf mines accounting for 11% (5 species). No association was detected with the morphological type of spiral mines.

Frass dependence on the host-plant family. The examination of the Nepticulidae mines showed that larvae mining various plant families leave frass of various colours. The predominant colour of frass, typical of 68 species, is black or blackish brown, whereas 25 species leave brown or reddish-brown frass. Leaf mines with green or reddish green frass are the least common (15 species). It must be noted that the numbers of species overlap: we found that in some cases the larvae of the same species left frass of different colours.

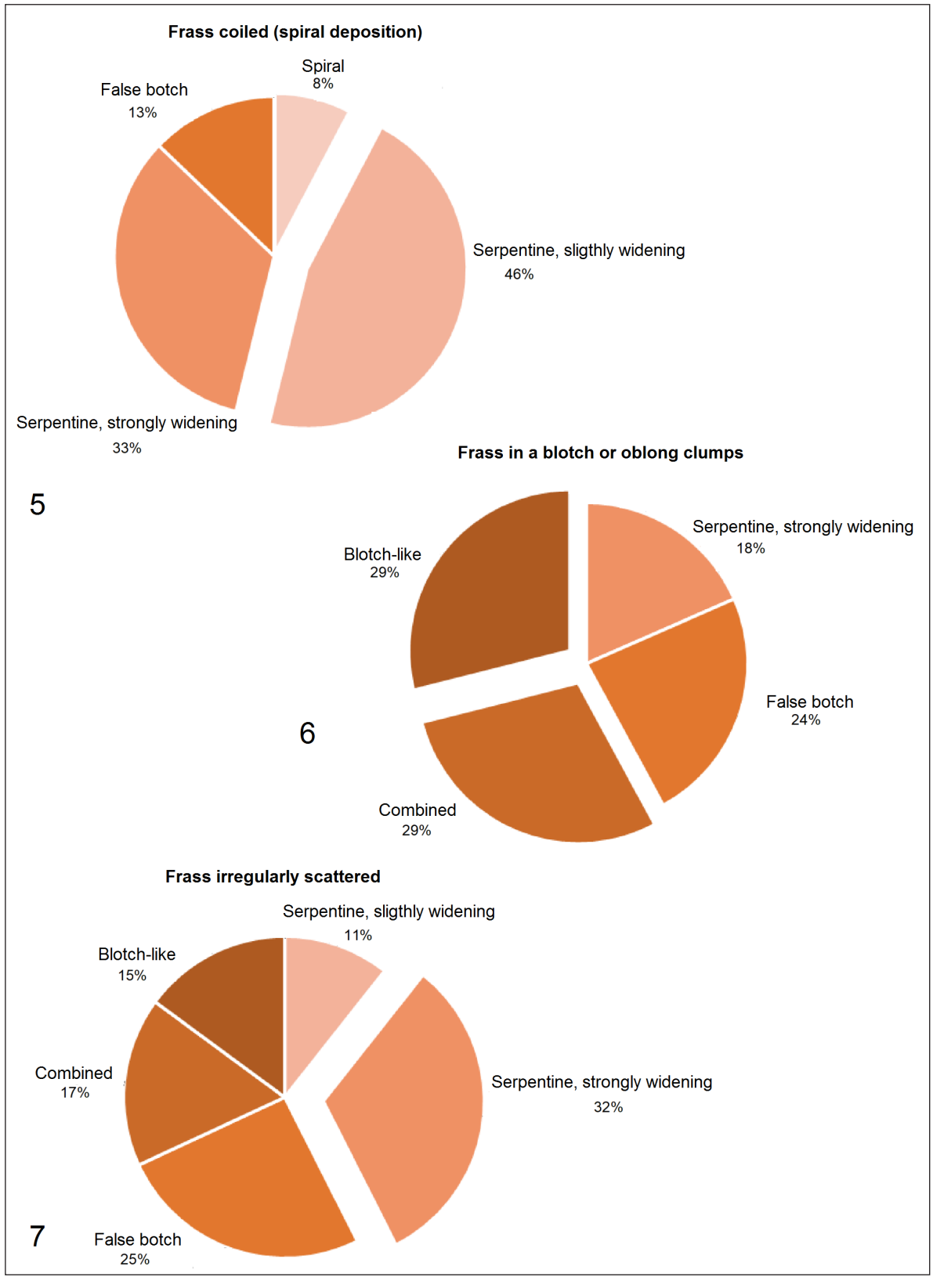
Special focus of the analysis was placed on the following seven host-plant families, which were the main plant families in the studied



Figs 1, 2. Initial part and the main part of a leaf mine. 1 – dependence of the filling of the initial part of the leaf mine with frass on the main part of the leaf mine; 2 – the data showing that when the main gallery had a wide line or band of frass and slender unfilled margins, often the initial part of the mine was also filled with frass



Figs 3, 4. Dependence of various frass deposition on the morphological type of the leaf mine



Figs 5–7. Dependence of various frass deposition on the morphological type of the leaf mine

sample of Nepticulidae: Betulaceae, Fagaceae, Ericaceae, Rosaceae, Salicaceae, Polygonaceae, and Sapindaceae. The purpose of the study was to determine whether the colour of frass depended on the systematic group of host plants.

The data showed that the black colour of frass was particularly common among the Nepticulidae species that mine Rosaceae (23 of such species). Twelve species of Nepticulidae mining Rosaceae leave brown frass and five species were identified as leaving green frass (Fig. 8). Among Betulaceae miners, species with black frass also predominates (12).

This study not only showed: (1) the predominance of species with black frass, (2) a weak relation between the colour of frass and the systematic position of host plants, but also revealed an unexpected exception among Nepticulidae: all species of the Salicaceae miners deposit only black frass. Nevertheless, it appears that the colour of frass is not related to the systematic position of the host-plant family or the taxonomic position of the miner itself. The study shows that among the different genera of Nepticulidae, there are species that leave frass of different colours (with black frass predominating). Moreover, there are species that are characterised by frass of different colours during their larval development. For example, the larvae of *Stigmella lapponica* (Wocke) leave green frass in the early stages of their development and deposit black frass at later stages. In the studied sample, the number of species that deposit frass of different colours despite feeding on the same host plant was relatively high: *Stigmella anomalella* (Göze), *S. centifoliella* (Zeller), *S. sorbi* (Stainton), *S. tiliae* (Frey), *S. freyella* (Heyden), *S. aceris* (Frey), *S. hemargyrella* (Kollar), *S. basiguttella* (Heinemann), and the earlier mentioned *S. lapponica* (Wocke). Again, this shows that the colour of frass has little (or usually none) relation to the systematic position of the host plant.

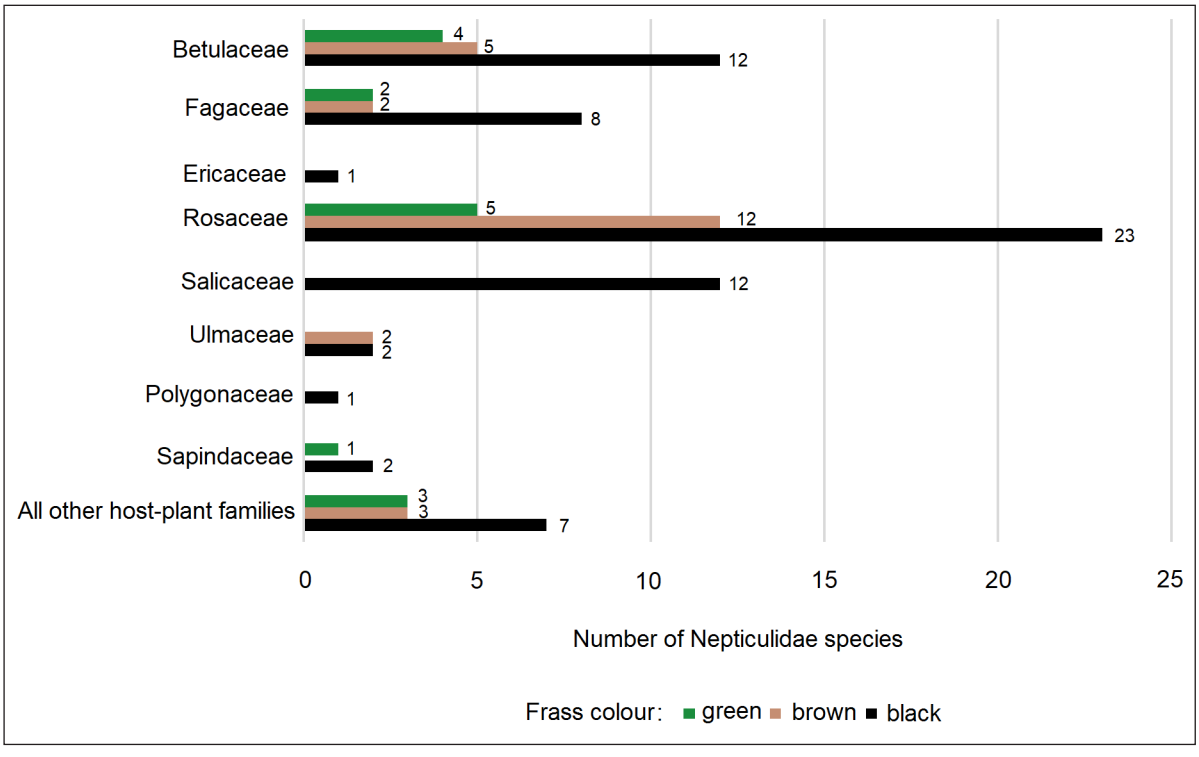


Fig. 8. Dependence of the frass colour on the host-plant family

DISCUSSION

In summing up the results of the study, it can be stated that filling the initial part of the leaf mine with frass is often related to frass deposition along the whole of the mine (the main gallery). The wider the frass band in the main gallery, the more likely it is that this mine will be completely filled with frass at the very beginning.

The study also suggests that the deposition of frass is related (at least partially) with different morphological types of leaf mines. Our study found that gradually widening leaf mines predominate among most of the mines with different frass deposition. Coiled (or spiral) frass is mostly characteristic of slender serpentine leaf mines, whereas the deposition of frass in patches or oblong clumps is mainly characteristic of blotch-like or combined leaf mines. Some correlation between frass deposition and morphological types of the mine suggests that ecological adaptations of drying frass were gained differently in different morphological types of leaf mines.

It also can be stated that frass colour has hardly any relation with the systematic group (i.e., family) of the host plant. In general, black frass is characteristic and even predominates among the miners of any host plant family and are particularly common among the Rosaceae miners (they account for 57.7% of all species with black frass). Moreover, all Salicaceae-miners are characterised by black frass. As for the groups of miners mining different host-plant families, the majority include species that leave frass of various colours (not only black). The only exception are the miners of plant families that are not numerous species-wise (e.g., Polygonaceae, Ericaceae, and partially Sapindaceae). Thus, black frass can be treated as a 'conservative' state of the character.

ACKNOWLEDGEMENTS

I am grateful to Jonas R. Stonis (State Research Institute Nature Research Centre, Vilnius, Lithuania) for the useful consultations and his other help during the preparation of this article.

Received 30 March 2022
Accepted 20 April 2022

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Viktorija Dobrynina

KAI KURIŲ NEPTICULIDAE MINŲ POŽYMIŲ PRIKLAUSOMYBĖS ASPEKTAI

Santrauka

Šiame straipsnyje nagrinėjamos kai kurių minų požymių sąsajos. Tyrimų rezultatai leidžia teigti, jog minos tako pradžios užpildymas ekskrementais yra sietinas su viso minos tako („pagrindinio“ tako) užpildymu ir kad ekskrementų išsidėstymą bent iš dalies lemia skirtingi minų morfologiniai tipai. Aptiktas ekskrementų išsidėstymo ryšys su minos morfologiniu tipu padeda geriau suvokti, kad skirtingas ekologinis prisitaikymas, susijęs su ekskrementų džiovinimu, priklausė nuo minos morfologinio tipo, o ekskrementų spalva menkai siejasi su mitybinių augalų sistematine grupe (t. y. šeima). Juodos spalvos ekskrementai yra būdingi bet kurių mitybinių augalų šeimų minuotojams.

Raktažodžiai: augalų minuotojai, lapų minos, Lepidoptera, mažieji gaubtagalviai, minologija