

THE WALNUT SHIELD BEARER *COPTODISCA LUCIFLUELLA* (CLEMENS, 1860) (LEPIDOPTERA: HELIOZELIDAE) – THE FIRST RECORD IN ROMANIA

Constantina Chireceanu,* Roxana-Violeta Mustăţea, Andrei Teodoru.

Research and Development Institute for Plant Protection, Laboratory of Harmful Organisms

*correspondence address

Research and Development Institute for Plant Protection Bucharest
Bd. Ion Ionescu de la Brad no. 8, CP 013813, Bucharest, Romania
E-mail: cchireceanu@yahoo.com

<http://www.doi.org/10.54574/RJPP.15.02>

Abstract: The invasive species *Coptodisca lucifluella*, also called the walnut shield bearer is recorded for the first time in Romania in this report. It is a leaf miner of North American origin, having been discovered for the first time in Europe (Italy) in 2010. Since then it has been confirmed in other three European countries, Hungary (2017), Ukraine (2019) and Bulgaria (2020), and now in Romania. In its native habitats, the species is known to be specialized on woody plants from the family Juglandaceae, genus *Carya*, in particular the pecan nut tree, *Carya illinoensis* (Wangenh.) K. Koch. In the newly occupied territories in Europe, the moth was reported mainly on *Juglans spp.*, especially *J. regia* (L.) (with very few instances on *J. nigra* (L.), *Carya spp.* and *Pterocarya spp.*). In August 2022, leaves of common walnut (*J. regia*) with mines of *C. lucifluella* were collected in Southern Romania (Bucharest, Ilfov and Giurgiu Counties) and Western Romania (Arad County). The walnuts were located in cities, rural areas, alongside roads and spontaneously grown in vineyards, at altitudes from 60 to 200 m a.s.l.

Key words: *Coptodisca lucifluella*, leaf miner moth, mines, common walnut, invasive species.

INTRODUCTION

The walnut shield bearer *C. lucifluella* is a micro leaf miner moth in the family Heliozelidae. A number of eight indigenous species and four invasive species belonging to this family are reported to be present in the European territory until now (Van Nieuwerkerken et al., 2018, Takács et al., 2020). *C. lucifluella* is one of the four invasive species, native to North America, together with *Antispila oinophylla* (Van Nieuwerkerken & Wagner, 2012), *C. splendoriferella* (Clemens, 1860) and *C. juglandiella* (Chambers, 1874) (Takács et al., 2020). *C. lucifluella* is native to the Eastern North America. In 2010 this species was recorded in Europe for the first time, in Italy, Region Campania (Bernardo et al., 2011). A more ample study of Bernardo et al., (2015) indicated a widespread presence of this species in many northern (Veneto) and southern (Basilicata) regions of Italy, being collected at all sampled localities with the exception of some trees situated at altitudes over 1200 m a.s.l. Poggetti et al., (2019) made further studies in Italy and found that the altitude is a determining factor in both the dispersal and abundance of the species, and that the insect is rarely found at 400 m and absent at more than 600 m.

In 2012, the species was signalled for the first time in Mexico in various pecan nut orchards (Ávila-Rodríguez et al., 2015) and in 2017, this is reported in Hungary (Takács et al., 2017). A more extensive survey in 263 localities dispersed throughout Hungary proves the vast spread of the species (Takács, et al., 2020). In 2019, *C. lucifluella* was identified in Ukraine in the proximity of the Hungarian border (Pályi et al., 2019). The most recent European report

indicating the presence of the insect is in Bulgaria, where it was found in 36 localities (Tomov, 2020). Distribution of the walnut shield bearer *C. lucifluella* in the world is listed in Table 1.

Table 1. World distribution of *C. lucifluella*

Continent/ Country	First recorded	Reference
North America		
United States (NE Texas, Mississippi, Georgia, North Carolina, Maryland, Kentucky, Pennsylvania, Ohio, Wisconsin, New York, Connecticut)	Native	Bernardo et al., 2015.
Mexico	2012	Ávila-Rodríguez et al., 2015.
Europe		
Italy	2010	Bernardo et al., 2011.
Hungary	2017	Takács et al., 2017.
Ukraine	2019	Pályi et al., 2019.
Bulgaria	2020	Tomov, 2020.
Romania	2022	The present report

This article presents the first report of the walnut shield bearer *C. lucifluella* in Romania.

MATERIALS AND METHODS

At the beginning of August 2022 our team started to look for the insect in Northern Bucharest (Southern Romania) (44°30'14.7"N 26°03'56.9"E, 89 m a.s.l.), in the proximity of the Research and Development Institute for Plant Protection (RDIPP). On 9 August 2022, the first leaves of common walnut with *C. lucifluella* mines were collected.

The discovery of walnut leaves bearing mines of this species motivated the search for other samples of *J. regia* trees in other green areas of Bucharest and also in the surroundings. A field survey was conducted in the didactic farm Moara Domnească (Ilfov County) situated at 15 km from the capital, which has a walnut orchard of approximately 800 trees. A second field survey was carried out in Giurgiu County (near Ilfov County), and a third survey took place in Arad County in Western Romania.

The walnut trees were observed, leaves with mines were collected, transported to the laboratory and analysed with the stereomicroscope Zeiss Stemi 508. The leaflets with both active mines and cases were placed in glass vessels and kept at room temperature conditions until the adults emerged. Fragments of walnut bark from the trunk and branches with insect cases were also placed in containers in order to obtain adults.

The species identification was performed following the morphological descriptions in the papers of Bernardo et al. (2015) and Takács et al. (2020). The images referring to different stages of this species as well as its attack were taken with camera Axiocam 105 color attached to the stereomicroscope. The material is deposited in the collection of RDIPP Bucharest.

Moreover, determinations were made in order to obtain the average percentage of leaves per tree with symptoms of attack and also the average number of mines per leaf. Firstly, samples of walnut leaves with symptoms of attack were collected from Bucharest and Moara Domnească

species of potential parasitoids, impossible to identify due to the fact that they were dry, deformed and with broken body parts. These parasitoids did not manage to get out of the mines.

The average number of mines on collected leaves that showed the symptoms of attack is as follows: on 10 August 59 leaves were collected, with an average number of 2.52 mines per leaf; on 11 August 234 leaves were collected, with an average number of 1.75 mines per leaf; on 18 August 104 leaves were collected, with an average number of 2 mines per leaf. Collection sites were Northern Bucharest and Moara Domnească.

On 19 August a survey was made in Moara Domnească on 12 trees, taking 50 random leaves per tree and counting the number of mines per leaf. This resulted in an average of 0.55 mines per leaf and an average of 42% attacked leaves per tree. The same survey was performed in Bucharest with 0.55 mines per leaf and an average of 40% attacked leaves per tree.

Morphological characteristics of the walnut shield bearer *Coptodisca lucifluella*

The adults (Figure 2a) and juvenile stages of *C. lucifluella* have been described by Bernardo et al. in 2015, after efforts of trying to find reliable studies in the literature of the lepidopterological fauna of North America and finding out that the species of the Heliozelidae are little studied and morphological characteristics described are hard to use in the identification of species within genera.

The male has the head, face and vertex covered with silvery scales. Antennae are silvery grey with 16 segments. The thorax and the basal third of the forewing is silvery white, turning darker towards the forewing posterior edge. Forewing is one-third yellow in costal area and two-thirds dark-almost black in the dorsal area, has intricate pattern of silver, black and yellowish spots, streaks and hairs. The wingspan is 4.1 ± 0.1 mm, the length of the forewing is 1.9 ± 0.2 mm with no difference between the sexes.

The female has the same colouring as the male. Antennae have 14 segments and the abdomen is distally pointed. The tip of the oviscapt has 5 teeth.

The larvae (Figure 3b) are yellowish-brown, the pre-pupal stages (Figure 5b) and newly formed pupae are pale yellow but darken with age. (Bernardo et al., 2015)

The egg has been poorly studied; it is inserted singly in the host's leaf tissue (Davis, 1998).

The mines have a blotch-like appearance (Figures 2b and 3a) and are visible from both sides of the leaf. They are always situated between lateral veins and never cross them. The general shape is angular and elongated. Blackish grains of frass can be observed dispersed inside the mine (Figure 3a and 3b), obvious from both sides of the leaflet (Takács et al., 2020). The colour is light grey – light brown. When completed have a mean length of 7.81 ± 0.211 mm and the mean width of 3.48 ± 0.104 mm. The mean number of mines per leaflet varies with the year and season and can have values between 0-29 (Bernardo et al., 2015).

The case is made by cutting small oval discs from the surface of the mine. Sometimes the case is not completely cut out from the mine (Figure 3c).

The case has a mean length of 2.92 mm and mean width of 1.74 mm. It is light brown and gets attached by fine white silk filaments on different surfaces – bark, leaves, twigs or lichens (Figure 4a, 4b, 4c and 4d). After the adult's emergence, the pupal exuviae can be seen extending out from the abandoned case (Figure 5a) (Bernardo et al., 2015).



Figure 2. a. Adult of the walnut shield bearer *C. lucifluella*; b. Blotch mines (active) on common walnut in Bucharest in August 2022; c. common walnut leaf with perforated (abandoned) mines.

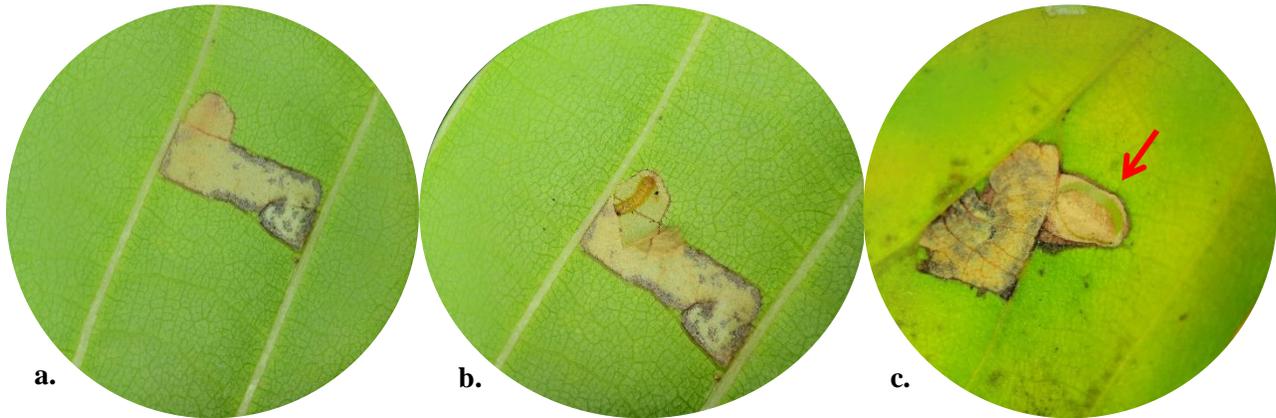


Figure 3. a. Active mine of *C. lucifluella* with larva hiding inside and blackish frass scattered on the margins; b. larva exposed from under the upper layer of the mine; c. constructed shield (case) of the walnut shield bearer not yet completely cut out from the leaf.

Life cycle

The females pierce the underside of leaves with their ovipositors where they lay eggs, a single one per orifice. The first instar larvae begin eating the mesophyll of the leaf, between the two epidermises, developing blotch-shaped mines with visible blackish frass grains which are retained inside. All larval instars mine except for the mature ones which create an oval-shaped cocoon (case, shield), by cutting the upper and lower epidermises of the mine, which they join with silk threads. Before pupation the larvae produce a silk strand to reach the ground, or they attach their cases to the bark or leaves of the host plant (Davis, 1998).



Figure 4. a. *C. lucifluella* active case attached to walnut bark; b. case attached to the underside of a walnut leaf; c. case fixed on a moss fragment; d. two active cases fixed on a walnut leaf.

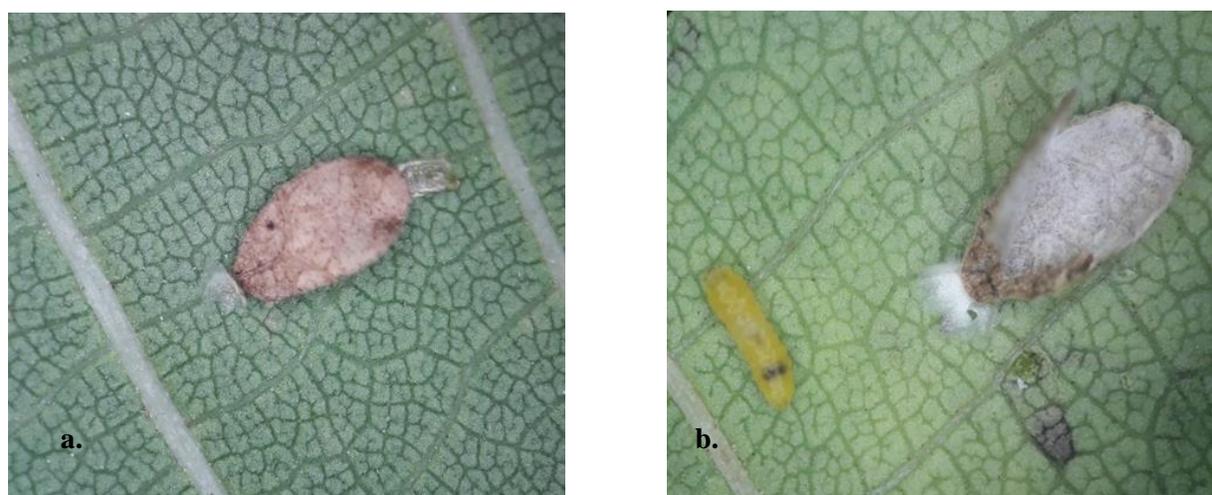


Figure 5. a. Case of *C. lucifluella* with pupal exuviae protruding attached by a common walnut leaf; b. yellow pre-pupal stage taken out of its case.

To reach the mature stage, a larva needs between eight and eighteen days. The pupal stage lasts between five and eight days (Takács et al., 2020). The pupal exuviae extend out from the cases after the adult's eclosion. The species has at least three or four generations per year in Italy (Bernardo et al., 2015). In Hungary the number of generations is not mentioned but the authors explain that active mines are continuously present on host plants from June to October, making it difficult to establish their real number (Takács et al., 2020).

C. lucifluella overwinters as a larva inside the cocoon, with pupation taking place the following spring or as an egg in warmer climates (Bernardo et al., 2015).

Damage

The main damage caused by this micro moth is represented by the abandoned mines with their characteristic circular holes (Figure 2c) (Davis, 1998). Through their mining, the surface area of the leaf is reduced. It was found that the percentage of infested leaves changes with the years, and also the locality (Bernardo et al., 2015) and altitude (Poggetti et al., 2019). In Italy, even a high percentage of infestation is not considered to have a serious economic impact on the walnut yield (Bernardo et al., 2015). Researchers in Hungary envision that a combination of unfavourable weather conditions along with a population explosion could lead to the untimely deterioration of walnut trees as a result of leaf surface loss (Takács et al., 2020).

Host plants

In its native range *C. lucifluella* is known to attack various species of *Carya* such as hickory and pecan nut trees (Payne et al., 1972; Davis, 1998; Bernardo et al., 2015; Takács et al., 2020). It is believed that because in Europe *Carya spp.* trees are scarcely planted - only in few ornamental gardens, *C. lucifluella* has shifted to *J. regia* as a preferred host. In this manner the species was able to survive but also to expand across the continent (Bernardo et al., 2011, Takács et al., 2020). Likewise in Italy, the leaf miner was found on *J. nigra* (Bernardo et al., 2011) and *Carya glabra* (Bernardo et al., 2015) and in Hungary on *C. cordiformis* and *Pterocarya fraxinifolia* (Takács et al., 2020).

In Romania at this stage of the research the leaf miner has been found only on *J. regia* trees.

In the near future, this insect species will be of particular importance for numerous walnut growers in the country. The walnut production has always been a traditional activity of high interest in Romania due to the nutritional value of the nuts and the economic importance of the wood. In 2020 alone 2.067.880 walnut trees were registered in the orchards (Soare et al., 2021). The walnut plantations are spread over a large area because of the favourable pedoclimatic characteristics found from the Black Sea to altitudes of 700-800 m, more commonly being cultivated in areas of semi-high hills at 250-400 m altitude (Iordănescu et al., 2021).

CONCLUSIONS

Based on data in this paper, we report that the walnut shield bearer *C. lucifluella* is a new invasive species which entered the territory of Romania and is spreading its distribution range in Europe.

It was collected from areas in the west and south of Romania on common walnut trees in cities, rural areas, alongside roads and on spontaneously grown walnut trees in vineyards, at altitudes from 60 to 200 m a.s.l.

We consider that *C. lucifluella* is widespread in the country and assume that it went unnoticed for an undetermined period of time before being detected in 2022.

As for the species' entry path, it is suspected that it was introduced with planting material because lately the walnut orchards are becoming more popular and planting material is imported from abroad. Another possibility is that the species migrated from neighbouring countries.

Therefore, further investigations are necessary in order to establish the species dispersal inside the country and more importantly some biological aspects such as the number of generations per year, its adaptability to climatic conditions and various host plants and also whether its populations are naturally kept in check by native parasitoids.

ACKNOWLEDGEMENTS

This study was conducted with internal sources of finance under the thematic planning of RDIPP 'Early detection of invasive insect species in Romania'.

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