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FIRST RECORD OF *NEODRYINUS TYPHLOCYBAE* (ASHMEAD) (HYMENOPTERA, DRYINIDAE) IN ROMANIA, A PARASITOID WASP OF THE CITRUS FLATID PLANTHOPPER *METCALFA PRUINOSA* (SAY)

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Abstract: *Neodryinus typhlocybae* (Ashmead 1893) (Hymenoptera, Dryinidae) is an important North American parasitoid wasp of the citrus flatid planthopper *Metcalfa pruinosa* (Say 1830), an invasive alien pest species accidentally introduced from North America to Europe in 1979. This has spread rapidly with large populations in many European countries, including in Romania since 2009. The planthopper is a polyphagous pest that strongly infests a wide range of plant species of agricultural, forestry and urban importance as well as spontaneous flora, in the absence of a specialized natural enemy or other control measures. Since 1987, the wasp *N. typhlocybae* was introduced into the N-E of Italy and released for the pest control. Other imports and targeted releases of the parasitoid wasp occurred in Croatia, France, Greece, the Netherlands, Slovenia, Spain and Switzerland. In addition to the deliberate introduction, *N. typhlocybae* has also spread naturally. Thus, this has also been reported in Austria, Hungary, Bulgaria and Slovakia. This paper reports the first record of *N. typhlocybae* in Romania. Leaves of various plant species attacked by *M. pruinosa*, collected from urban area of Bucharest in September 2019, showed the presence of the wasp cocoons. Most cocoons were recorded on the leaves of *Morus sp.* and *Mahonia aquifolium*. It can be assume that the penetration of *N. typhlocybae* in Romania occurred naturally from Bulgaria.

Key words: *Neodryinus typhlocybae, Metcalfa pruinosa*

INTRODUCTION

In 1979, the citrus flatid planthopper *Metcalfa pruinosa* (Hemiptera, Flatidae) was accidentally entered Europe through Italy, the province of Treviso (Zangheri & Donadini, 1980). Soon after, this invasive planthopper species rapidly spread to other European countries. In Romania it was first reported in 2009 (Preda & Skolka, 2009) in the south part of the country and in the present is currently found all over the country (Ciceoi et al., 2017). *M. pruinosa* is a highly polyphagous species, strongly attacking countless species of plants from different botanical groups, wild and of economic importance. Consequently, an effective control strategy against this species has become mandatory. In this purpose, the Nearctic parasitoid wasp *Neodryinus typhlocybae* (Ashmead 1893) (Hymenoptera, Dryinidae) has been imported in Europe from the USA in the late eighties as part of an elaborate biological control program against *M. pruinosa*. *N. typhlocybae* is known the only natural enemy of *M. pruinosa* that occurs in the native range of *M. pruinosa* in North American (Girolami & Mazzon, 2001). The parasitoid was successfully released first in N-E Italy in 1987 and then in other European countries such as Switzerland, Slovenia, Croatia, France, Spain, Greece, the Netherlands (Strauss, 2012; Vetek et al., 2019). Presence of the parasitoid was also reported in other European countries where it was not deliberately introduced. Clare evidence is recent reports of Szöllősi-Tóth et al. (2017) for Hungary, of Lapeva Gjonova et al. (2018) and Tomov & Vasileva (2018) for Bulgaria and of Vetek et al., 2019 for Slovakia. It is assumed

that the species entered naturally from neighbouring countries with them, like Austria, Croatia and Slovenia (Vetek et al., 2019) when the wasp already has stable populations.

This paper presents the first data on the presence of the parasitoid wasp *N. typhlocybae* in Romania (urban area of Bucharest, south part of the country) in *M. pruinosa* colonies.

MATERIALS AND METHODS

Between 10 and 17 September 2019, leaves of various plant species with attack of *M. pruinosa* and bearing cocoons of the parasitoid wasp *N. typhlocybae* were collected in the northern part of Bucharest, the cocoons being attached to the exuviae of *M. pruinosa* nymphs on the lower or upper surface of leaves. Three sites with semi natural vegetation, ornamental plants and shrubs have been used for sampling: an abandoned field, an experimental field and the public park Herastrau, all placed in a five square Km area. Leaves covered with honey dew, white waxy secretions and nymph exuviae, signs specific to infestation of *M. pruinosa* pest have been observed. Cocoons of parasitoid wasp look like as silky white halos of oval or circular shape among the waxy secretions and exuviae of the pest. Plant species identification and counting the wasp cocoons per leaf were done both in the field and in the lab. Some of cocoons were studied under a stereomicroscope SZ 61 following the morphological characteristics and illustrations in the literature which describe the wasp in the larvae and adult form. To obtain adults of *N. typhlocybae*, leaves with cocoons, sampled at the three sites, were stored in glass jars covered with cloths at ambient temperature in laboratory.

RESULTS AND DISCUSSION

During the investigation of the leaves of different plant species in urban area of Bucharest in September 2019, there were recorded colonies of *M. pruinosa* nymphs' exuviae in association with cocoons of the parasitoid wasp *N. typhlocybae*. The results of observations and recordings related to the presence of the cocoons of *N. typhlocybae* are presented in the table 1.

Through these results, the presence of the *N. typhlocybae* in Romania is certified. This finding was to be expected, having into account the assessments for Hungary, Bulgaria and Slovakia, where it was estimated that the wasp naturally arrived from neighbouring countries (Vetek et al., 2019).

Table 1. *Neodryinus typhlocybae* cocoons on leaves of different plant species recorded in Bucharest area in September 2019

	Plant species	No. of cocoons/ leaf	Sites	Date
1	*Parthenocissus quinquefolia* (Virginia creeper)	2	abandoned field	10.09.2019
2	*Juglans regia* (English walnut)	1	abandoned field	10.09.2019
3	*Morus sp.* (Mulberries)	1-6	abandoned field	10.09.2019
4	*Prunus mahaleb* (Mahaleb cherry)	2	abandoned field	10.09.2019
5	*Prunus cerasifera* (Cherry plum)	1-2	abandoned field	10.09.2019
6	*Acer platanoides* (Norway maple)	1-4	abandoned field	10.09.2019
7	*Ligustrum vulgare* (European privet)	1	Herăstrău park	16.09.2019
8	*Sorbus sp.*(Rowan)	1	Herăstrău park	16.09.2019
9	*Jasminum sp.* (Jasmine)	1	Herăstrău park	16.09.2019
10	*Mahonia aquifolium* (Oregon grape)	1-6	experimental field	17.09.2019
11		1-5	abandoned field	18.09.2019
12	*Physocarpus opulifolius* (Common ninebark)	1	experimental field	17.09.2019

13	*Cornus mas* (European cornel)	1-2	experimental field	17.09.2019
14	*Tilia sp.* (Lime tree)	1	experimental field	17.09.2019
15	*Ziziphus jujuba* (Chinese date)	1	experimental field	17.09.2019

The number of cocoons per leaf ranged between 1 and 6 depending on the sampled plant species. The highest number of six cocoons on a leaf was recorded for plants of the genus *Morus* and the species *Mahonia aquifolium*. Only 1 cocoon per leaf was recorded in case of *Juglans regia*, *Ligustrum vulgare*, *Sorbus sp.*, *Jasminum sp.*, *Physocarpus opulifolius*, *Tilia sp.* and *Ziziphus jujube*.

It was also observed that the larger colonies of nymphs of *M. pruinosa* on the leaves contained a larger number of parasitic cocoons (Figures 1 and 2).

Figure 1. Five *N. typhlocybae* cocoons among *M. pruinosa* dead nymphs on *Mahonia aquifolium*

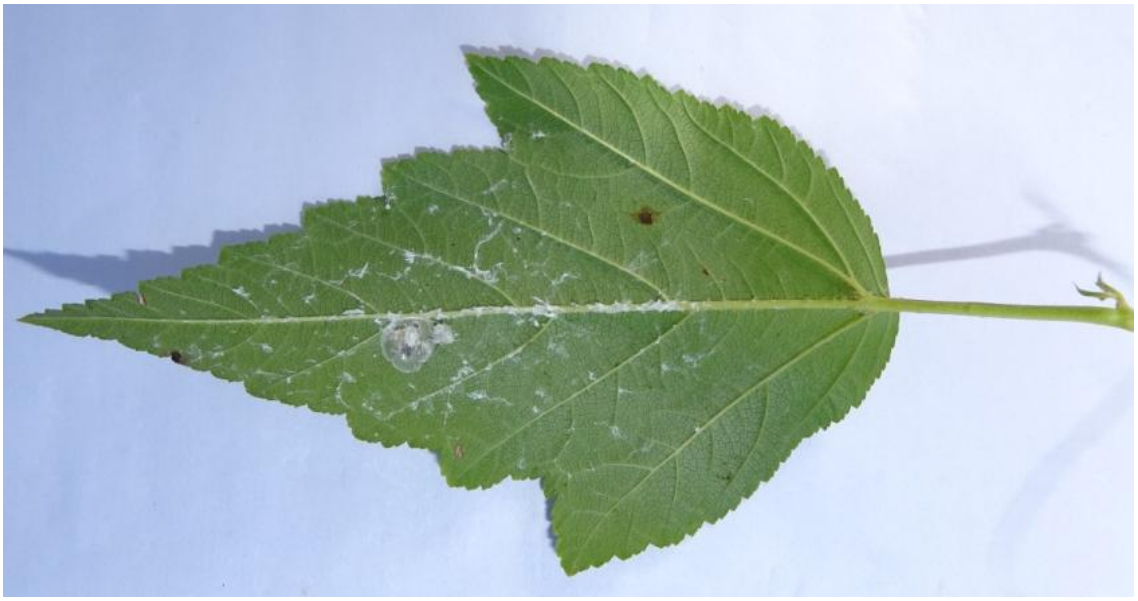

Figure 2. One *N. typhlocybae* cocoons and *M. pruinosa* nymph's exuviae on *Physocarpus opulifolius*

Most of the cocoons had on top of them residues of the dead host nymphs of *M. pruinosa* (Figure 3).

Figure 3. *N. typhlocybae* cocoon under the residue of a *M. pruinosa* nymph

In the laboratory under stereomicroscope, after gently opened some of the cocoons, live larvae of *N. typhlocybae* were observed inside (Figure 4).

Figure 4. *N. typhlocybae* larvae in and out of the cocoon

As for the leaves with cocoons of *N. typhlocybae* maintained at ambient temperature in the laboratory to obtain adults, at a checking after a period of 2-3 weeks, it was observed the imago of the wasp formed in the cocoons, but no adults emerged. We observed that some of larvae in the cocoons continued to develop and transform in imago (Figure 5) but these remained inside cocoons, and others which stayed at the larval stage. According to Vetek et al. (2019), there are two distinct fractions in the wasp *N. typhlocybae* population, following different destinies, one fraction develops one generation per year and the other fraction produces two generations per year. Interpreting our observation according to Vetek et al. (2019) findings, the larvae in the cocoons which were not transformed in adults, entering diapauses, belong to the univoltine fraction of the parasitoid wasp, while the imago in the cocoons are from the bivoltin fraction of the wasp. In Hungary, individuals from bivoltine fraction were in a higher proportion of than those from univoltine fraction (1: 0.18-0.56) (Vetek et al. 2019). This aspect was not measured during our observation, which is why it will be addressed in the further investigation.

Figure 5. *N. typhlocybae* imago incompletely developed outside the cocoon

CONCLUSIONS

Through the sampling results obtained in the field survey in the year 2019, the presence of the parasitoid wasp *Neodryinus typhlocybae* was confirmed in Romania, Bucharest urban area. This represents the first record of the species in the country.

As in the case of the countries nearby Romania, namely Hungary and Bulgaria, the species entered naturally as no biological control program performed here.

We presume that the parasitoid entered the southern part of Romania through Bulgaria mainly because it was reported there in 2018 and the city of Bucharest is relatively close to the northern Bulgarian border.

It is important in the near future to evaluate the parasitoid impact on the invasive planthopper species *Metcalfa pruinosa* that is its primary host in European countries.

Another important aspect that needs to be followed is the identification of the potential hyperparasitoids of *N. typhlocybae* in Romania and their negative impact.

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