Neopulvinaria innumerabilis (Rathvon, 1854), a new species of Coccidae in Serbia

Marina Dervišević¹* and Draga Graora²

¹Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia

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SUMMARY

In 2016, the species *Neopulvinaria innumerabilis* (Rathvon, 1854), belonging to the scale insect family Coccidae, was for the first time found in Serbia, in the location Radmilovac on *Cornus sanguinea* L. It reproduces by gamogenesis, develops one generation annually, and overwinters as a fertilized female on the trunk or branches of its host plant. During this research, the species was detected on five host plants at six locations in Serbia. It feeds by sucking sap from leaves, young shoots and branches of infested plants, causing desiccation of leaves and individual twigs. Four species of its natural enemies were collected and reared from colonies of *N. innumerabilis*. Two species of parasitoid wasps, *Coccophagus lycimnia* (Walker) (Aphelinidae) and *Metaphycus hageni* Daane and Caltagirone (Encyrtidae), were found, while the ladybird *Exochomus quadripustulatus* L. (Coccinellidae) and the fly *Leucopomyia silesiaca* (Egger) (Chamaemyiidae) were identified as predators. The most abundant entomophagous species was *L. silesiaca*.

Keywords: cottony maple scale, Coccidae, natural enemies, Serbia

INTRODUCTION

The cottony maple scale, *Neopulvinaria innumerabilis* (Rathvon), is an insect belonging to the family Coccidae (Hemiptera: Coccomorpha). The species is native to North America from where it was likely introduced to many European countries through plant material transport. It was first recorded in France (Cannard, 1966), then in Italy (Pellizzari, 1977), Azerbaijan, Armenia, Georgia, Russia (Hadzibeyli, 1983; Hodgson, 1994), Slovenia (Seljak, 1995) and Croatia (Masten-Milek, 2007). The species is polyphagous and infests over 50 plant species from 29 genera (García-Morales

et al., 2016), and it is found as an important pest in vineyards in North America (Gill, 1988), France and Italy (Pellizzari, 1977; Pellizzari & Germain, 2010), Croatia (Masten-Milek, 2007) and Slovenia (Štrukelj et al., 2012). It causes damage by direct feeding, which leads to stunted growth, premature leaf fall, as well as reduced quantity and market value of plants (Ülgentürk & Ayhan, 2011). It is also a vector of viruses from the family Closteroviridae, including Grapevine leafroll viruses (GLRaV-1, GLRaV-3) and Grapevine virus A (GVA) in vines (Sforza et al. 2003; Herrbach et al., 2017).

In 2016, Neopulvinaria innumerabilis was detected for the first time in Serbia at the location Radmilovac

²University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia

^{*}Corresponding author: marina.dervisevic@pesting.org.rs

on *Cornus sanguinea* L., which initiated a more detailed study of this species. The current paper presents the results of studies on its distribution, damage that it causes, morphological characteristics and development cycle, as well as the natural enemies of this scale pest in Serbia.

MATERIAL AND METHODS

The current study of the life cycle of *N. innumerabilis* was carried out on *Cornus sanguinea* L. at the location Radmilovac during 2016 and 2017.

N. innumerabilis presence, distribution, intensity of infestation and symptoms of damage that it causes were determined by visual inspection of plants and sampling of infested plant material. Infestation intensity was determined using the Borchsenius (1963) scale. Plant material was sampled every 7-10 days during vegetation, and once a month during vegetative rest. Samples of five one- or two-year-old twigs, 20 cm long, were taken from each infested plant.

In the laboratory, we examined the sampled plant material, reared and made permanent microscopic slides of soft scales, and identified the scale and its natural enemies.

To analyze the morphological characteristics of the scale, permanent microscopic slides of females were made following a method developed by Kosztarab & Kozár (1988), and identification was performed using the identification keys of Gill (1988) and Kosztarab & Kozár (1988).

For rearing purposes, the sampled twigs with scale colonies were placed in glass cylinders covered with dense synthetic meshes. The time of oviposition, number of laid eggs, and duration of embryonic and postembryonic development of scales were monitored by daily inspection of twigs. The average number of eggs laid by females was determined by counting the eggs of 10 females.

The plant material with scale colonies was placed in glass cylinders for rearing parasitoids. They were checked daily to determine the time and number of eclosed parasitoid specimens. Wasps were collected using a mouth aspirator, killed with ethyl acetate, mounted on cards and identified by Aleksandar Stojanović (Natural History Museum, Belgrade, Serbia). The mounted specimens are preserved in the Laboratory for Entomology and Agricultural Zoology, Faculty of Agriculture, University of Belgrade, Serbia.

Determination of ladybird species (Coleoptera: Coccinellidae) was carried out using the key of Bieńkowski (2018), while predatory flies (Diptera:

Chamaemyiidae) were determined by Radoslava Spasić (University of Belgrade, Faculty of Agriculture, Serbia).

RESULTS

Based on female morphological characteristics, the species was identified as *Neopulvinaria innumerabilis* (Rathvon), a member of the family Coccidae.

Morphological characteristics of *N. innumerabilis*

The female of *N. innumerabilis* is 3-10 mm long. It is light yellowish-brown with purple patterns on dorsal surface (Figure 1). Female antennae are 8-segmented. Its legs are very small compared to the body. Sclerotization is noticed between the tarsus and tibia. Marginal setae are spine-like, with blunt tips. Stigmal setae are present, three in each group. Body setae are spine-like and scattered over the entire body. Submarginal glands are absent. Simple discoid pores are distributed all over the dorsal surface. Quinquelocular pores are present in the area of stigmas. Multilocular pores (10 loculi) form transverse rows around the vulva. There are three types of tubular glands on the body. As the female matures, its skin on the dorsal surface becomes slightly sclerotized.

N. innumerabilis life cycle

During this research, N. innumerabilis was found to reproduce by gamogenesis, develop one generation annually, and overwinter as a fertilized female on the trunk or branch of its host plant. In the spring, females continue development, forming ovisacs (Figure 2) to lay eggs into. Oviposition was recorded in the second half of May (Table 1). The average number of eggs laid per female was 998.3 \pm 5.6 (Figure 3). Embryonic development lasts between two and three weeks before first-instar nymphs hatch. The hatched nymphs actively move around the plant, searching for suitable feeding site. They mostly concentrate on the underside of leaves where they feed by sucking plant sap. Second-instar nymphs developed by the end of July (Figure 4). During this period, sexual differentiation becomes noticeable. The larvae of future females developed until the end of August 2016, and early September 2017, when they molted and became females. The larvae of future males briefly fed before going through prepupal and pupal stages. Prepupae were observed in the first ten days of August 2016 and in the second decade of August 2017. Pupae were found in the second decade of August 2016 and third decade of August 2017. The prepupal and pupal stages lasted 10-12 days each, after which males hatched. Their appearance is synchronized with the appearance of females, and they flew in late August or early September. The sex ratio was 0.9. After copulation males die and fertilized females descend to the trunk or thicker branches to overwinter.

N. innumerabilis distribution, host plants, infestation intensity and damage symptoms

After its first detection at Radmilovac, *Neopulvinaria innumerabilis* was detected at five more locations in Serbia (Bečmen, Košutnjak, Kragujevac, Ušće, Zemun Polje). Infestation was recorded on five plant species from five botanical families (*Cornus sanguinea L.* [Cornaceae] *Maclura pomifera* [Raf.] Schneid. [Moraceae]; *Acer*

pseudoplatanus L. [Sapindaceae]; Ulmus minor Mill. [Ulmaceae] and Vitis vinifera L. [Vitaceae]) (Table 2).

Two plant species, *Maclura pomifera* and *Ulmus minor*, were found for the first time to be hosts of *N. innumerabilis*, which represents a new finding in the world literature.

N. innumerabilis scales found on Vitis vinifera at Bečmen and Ušće, and on Maclura pomifera at Košutnjak, formed dense colonies with infestation intensity 4 (Figures 5-6). Larvae feed by sucking sap from leaves, young shoots, and branches, causing leaves and individual twigs to dry. In the spring, infested plants are readily noticed owing to large scales with white ovisacs covering plant branches.

On *Acer pseudoplatanus* at the location Kragujevac, *Cornus sanguinea* at Radmilovac, and *Ulmus minor* at Radmilovac and Zemun Polje, *N. innumerabilis* was found sporadically or in small colonies with infestation intensity 3 and 4 and no visible symptoms of damage.

Table 1. The life cycle of *N. innumerabilis* on *Cornus sanguinea*

| Year | 2016 | | 2017 | |
|-------------------|--------------------|------------------|--------------------|------------------|
| Development stage | Female development | Male development | Female development | Male development |
| egg | 20.05. | 20.05. | 22.05. | 22.05. |
| N_1 | 07.06. | 07.06. | 11.06. | 11.06. |
| N_2 | 24.07. | 24.07. | 29.07. | 29.07. |
| prepupa | - | 07.08. | - | 12.08. |
| pupa | - | 18.08. | - | 22.08. |
| male | - | 28.08. | - | 04.09. |
| female | 30.08. | - | 06.09. | - |

 N_1 – first-instar

Table 2. Distribution, host plants, and infestation intensity of N. innumerabilis

| Location | Host plant | Infestation intensity | |
|-------------|----------------------------------|-----------------------|--|
| Bečmen | Vitis vinifera | 4 | |
| Košutnjak | Maclura pomifera* | 4 | |
| Kragujevac | Acer pseudoplatanus | 2 | |
| Radmilovac | Cornus sanguinea Ulmus minor* | 2 2 | |
| Ušće | Vitis vinifera | 4 | |
| Zemun Polje | Ulmus minor | 2 | |

^{*} Host plants on which N. innumerabilis was detected for the first time

N2 - second-instar



Figure 1. Female of *N. innumerabilis* (orig.)



Figure 2. Female of *N. innumerabilis* with ovisac (orig.)



Figure 3. Eggs of *N. innumerabilis* (orig.)



Figure 4. Second-instar nymph of *N. innumerabilis* (orig.)



Figure 5. Colony of N. innumerabilis on V. vinifera (orig.)



Figure 6. Colony of N. innumerabilis on M. pomifera (orig.)

| Table 3. | Natural | enemies of N. innumeral | hilis |
|----------|---------|-------------------------|-------|
| | | | |

| Order | Family | Species | Location | Total eclosed individuals |
|-------------|---------------|-------------------------------|-------------|---------------------------|
| Hymenoptera | Aphelinidae | Coccophagus lycimnia (Walker) | Zemun polje | 4 |
| | Encyrtidae | Metaphycus hageni Daane | Košutnjak | 2 |
| | | and Ĉaltagirone | Radmilovac | 1 |
| Coleoptera | Coccinellidae | Exochomus quadripustulatus L. | Radmilovac | 1 |
| Diptera | Chamaemyiidae | Leucopomyia silesiaca (Egger) | Radmilovac | 25 |

Natural enemies of N. innumerabilis

Four species of natural enemies were collected from *N. innumerabilis* colonies and reared, two of which were parasitoids of the order Hymenoptera, and the other two were predators of the orders Coleoptera and Diptera (Table 3).

Specimens of the parasitoid species *C. lycimnia* and *M. hageni* were found at three locations (Zemun polje, Košutnjak and Radmilovac). *C. lycimnia* parasitizes second instar larvae and females, while *M. hageni* parasitizes only females of *N. innumerabilis*. The predatory species *E. quadripustulatus* and *L. silesiaca* were recorded at Radmilovac. A total of 25 adults of the predatory fly *L. silesiaca*, whose larvae feed on scale eggs laid in egg sacs, were reared. Between one and four larvae of the predator completed their development in each ovisac of *N. innumerabilis*. The species *E. quadripustulatus* was represented by a single specimen.

DISCUSSION

N. innumerabilis was first recorded in Serbia at the location Radmilovac, on Cornus sanguinea in 2016. It reproduces by gamogenesis and develops one generation annually. Fertilized females overwinter on branches or trunks of their host plant, which coincides with data presented by other authors (Hadzibeyli, 1955; Phillips, 1962; Canard, 1966; Seljak, 1995; Pellizzari, 1997; Ülgentürk & Ayhan, 2011). In the spring, females become active, continue feeding, and begin laying eggs at the end of May. In our research, one female was found to lay up to 1000 eggs into a white ovisac, while other authors have reported the number of eggs to be as high as 3000 (Hadzibeyli, 1955) or even 8000 (Cannard, 1966). After hatching, mobile larvae move over the plant and feed intensively after finding a

suitable site to settle down. Females and males develop at the beginning of autumn. After copulation, males die and fertilized females overwinter. Similar data on the life cycle were reported by Gill (1988) and Štrukelj (2012).

N. innumerabilis was in this research found on five plant species at six locations. To date, it has been recorded on over 50 host plants, including a large number of fruit trees and ornamental plants (García-Morales et al., 2016). Regarding ornamental plants, Maclura pomifera and Ulmus minor were identified as new hosts of N. innumerabilis.

The scale forms colonies of variable size on infested plants. Numerous colonies were discovered on grapevines at the locations Bečmen and Ušće, as well as on *Maclura pomifera* in Košutnjak. Symptoms of drying and premature leaf fall were observed on these plants.

Neopulvinaria innumerabilis prefers grapevines, to which it is able to cause significant economic damage (Seljak, 2007; Pellizzari, 1997). Due to feeding by larvae and females, plants become physiologically weakened, shoots are thinner, and leaves are smaller. Additionally, the species secretes honeydew on which sooty mold fungi settle, covering leaves and fruits, which ultimately reduces yield and market value of grapes (Seljak & Žežlina, 2007). Similar damage has been observed on ornamental plants in Georgia (Pellizzari, 1997).

Four entomophagous species originating from colonies of *N. innumerabilis* were reared, of which *C. lycimnia* and *M. hageni* are parasitoids, while *E. quadripustulatus* and *L. silesiaca* are predators. All recorded natural enemies are present in small numbers. *Metaphycus hageni* is a recent addition to the fauna of Serbia, and *N. innumerabilis* is a new host for this parasitoid wasp (Dervišević et al., 2024b). It has only been reported in global literature as a parasitoid of olive black scale *Saissetia oleae* (Olivier) (Daane et al., 2000). *Coccophagus lycimnia* is often

found in colonies of scale insects. In Serbia, it has been recorded on 16 species (Mitić-Mužina, 1964; Mihajlović & Kozarževskaja, 1983; Dervišević et al., 2024a), while over 100 species in the family Coccidae host it worldwide (Noyes, 2019).

The predatory ladybird *E. quadripustulatus* has been recorded in Serbia on species in the family Coccidae (Mitić-Mužina, 1964; Graora et al., 2012; Simonović et al., 2018; Dervišević et al., 2024a) and Diaspididae (Mihjalović & Kozarževskaja, 1983). It is considered an effective predator of scale insects of the families Coccidae, Diaspididae, Eriococcidae, and Kermesidae (García-Morales et al., 2016). The predatory fly *L. silesiaca* has been recorded in Serbia on *Pulvinaria vitis*, *P. hydrangeae*, and *P. flocifera* so far (Graora et al., 2016), while on *N. innumerabilis* it has been recorded only recently (Dervišević et al., 2024a). In Europe, *L. silesiaca* is reported as a predator of numerous species in the families Coccidae, Pseudococcidae, and Eriococcidae (Teodorescu & Maican, 2014).

Low abundance of natural enemies is probably due to the low abundance of scale populations on most infested plants. Considering this is a new species in Serbia, further research should focus both on studying *N. innumerabilis* and on studying the role and importance of its natural enemies.

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Neopulvinaria innumerabilis (Rathvon, 1854), nova vrsta štitaste vaši u Srbiji

REZIME

Tokom 2016. godine u lokalitetu Radmilovac na *Cornus sanguinea* L., prvi put je utvrđena vrsta *Neopulvinaria innumerabilis* (Rathvon, 1854), koja pripada štitastim vašima iz familije Coccidae. Razmnožava se gamogenezom, razvija jednu generaciju godišnje i prezimljava u stadijumu oplođene ženke na stablu ili granama domaćina. Tokom istraživanja vrsta je u Srbiji registrovana na šest lokaliteta i pet biljaka domaćina. Na infestiranim biljkama hrani se isisavanjem sokova iz lišća, mladih izbojaka i grana, izazivajući sušenje listova i pojedinačnih grančica. U kolonijama *N. innumerabilis* sakupljene su i odgajene četiri vrsta prirodnih neprijatelja. Od parazitoida, *Coccophagus lycimnia* (Walker) (Aphelinidae) i *Metaphycus hageni* Daane and Caltagirone (Encyrtidae), a od predatora *Exochomus quadripustulatus* L. (Coccinellidae) i *Leucopomyia silesiaca* (Egger) (Chamaemyiidae). Najbrojnija entomofagna vrsta je *L. silesiaca*.

Ključne reči: javorova štitasta vaš, Coccidae, prirodni neprijatelji, Srbija