

AREA EXTENSION OF *DIPLOLEPIS ROSAE* (CYNIPIDAE) IN THE WESTERN REGION OF ROMANIA

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Abstract. *Diplolepis rosae* (Hymenoptera: Cynipidae) is a monophagous species (attacks species of the genus *Rosa*, family Rosaceae), monovoltine with uncertain origin, though assigned as an ancestral area is the eastern Mediterranean. The species is present in North America, Asia, Australia - Oceania and Europe. In Romania, the species was first reported in 2008, in Cluj County. The purpose of this study is to report the species *Diplolepis rosae* in the western part of Romania and to describe the damage caused. The study began in 2019, in the locality of Giarmata Vii, when galls were observed on the plants of *Rosa canina* (Rosaceae) produced by the species mossy rose gall wasp. These observations were decisive in conducting the study, in 2020 additional research were carried out in respect of the monitoring and mode of damage caused by mossy rose gall wasp to *Rosa canina* plants. The distribution area of the species *Diplolepis rosae*, in the western part of Romania, was established through observations in situ in the counties: Timiș, Arad and Caras-Severin. To describe the damage, mature galls were harvested in October-April, then transported and analyzed in the laboratory. These galls are the result of physiological changes of the host plants, inside them larvae develop. Studies show that galls have different sizes that are closely correlated with the number of lodges and larvae/galls.

Keywords: *Diplolepis rosae*, *Rosa canina*, galls, area, reporting

INTRODUCTION

The family Cynipidae is represented by about 1500 species (GUTIÉRREZ ET. AL, 2021), these can be found from the temperate zone to the northern hemisphere (GAMZE and YUSUF, 2020). The Cynipidae family includes 12 tribes: Paraulacini, Phanacidini, Pediaspini, Qwaqwaiini, Synergini, Aylacini, Aulacideini, Eshatocerini, Ceroptresini, Cynipini, Diastrophini și Diplolepidini ((RONQUIST ET AL. 2015; GAMZE and YUSUF, 2020). In Europe, the Cynipidae family is represented by about 300 species (NIEVES-ALDREY, 2001a; MELIKA 2006; ABE ET AL. 2007; GAMZE and YUSUF, 2020). The *Diplolepidini* tribe comprises two genera, *Diplolepis* and *Liebelia*, having host specificity, producing galls only to plants belonging to genus *Rosa* L. (GUTIÉRREZ et. al, 2021).

Most species, from the Cynipidae family, have as host plants: species from *Fagaceae* (86%), 7% plants belonging to *Rosa* L. și 7% grassy or woody species in families: *Rosaceae*, *Asteraceae*, *Lamiaceae*, *Papaveraceae*, *Valerianaceae* și *Aceraceae* (NIEVES-ALDREY, 2001a; GUTIÉRREZ ET. AL, 2021; OLTEAN ET AL., 2015).

The genus *Diplolepis* comprises 50 species (GUTIERREZ ET. AL, 2021) including *Diplolepis rosae*, which is the subject of this study. The presence of the species has been reported on the continents: Africa, Asia, North America, Australia and Europe (URBAN, 2018, CABI).

In Europe, according to literature, the species *Diplolepis rosae* is present in: Austria (KOHNE, 2009), Bulgaria (TODOROV, 2012), Czech Republic (URBAN, 2018) Denmark (KOHNE, 2009), Germany (KOHNE ET AL., 2011), Greece (CABI Undated), France (KOHNE, 2009), Macedonia (KOHNE, 2009), Great Britain (CABI Undated, KOHNE, 2009),

Serbia (MARKOVIĆ, 2015), Ukraine (MELIKA, 2006), Malta (MIFSUD, 2016), Slovakia (KOLLÁR, 2007), Spain (CABI Undated, KOHNEN, 2009, GUTIÉRREZ ET AL., 2021), Sweden (CABI Undated, STILLE, 1984), Hungary (LÁSZLÓ and TÓTHMÉRÉSZ, 2006, 2011, 2019; KOHNEN A., 2009), Ireland (O'CONNOR, 2004), Italy (RIZZO and MASSA, 2006), Finland (SHOHREH DANESHVAR ET AL., 2009), Netherlands (SHOHREH DANESHVAR ET AL., 2009), Norway (KOHNEN, 2009) Poland (SHOHREH DANESHVAR ET AL., 2009).

Information on the number of host plants for *Diplolepis rosae* are uncertain. Studies conducted by DARBOUX și HOUARD (1901), suggests that the species *Diplolepis rosae* is found on about twenty species of *Rosa* (URBAN, 2018). Studies of KOVALEV (1981), GAUSS (1982), STILLE (1984), ELLIS (2004), O'CONNOR (2004), BOWDREY (2007), SYRETT (1990), LIU ZHANG and XIAO (2012) reported *Diplolepis rosae* on the species shown in figure 1. Most studies and reports of mossy rose gall wasp refer to the species *Rosa canina*.

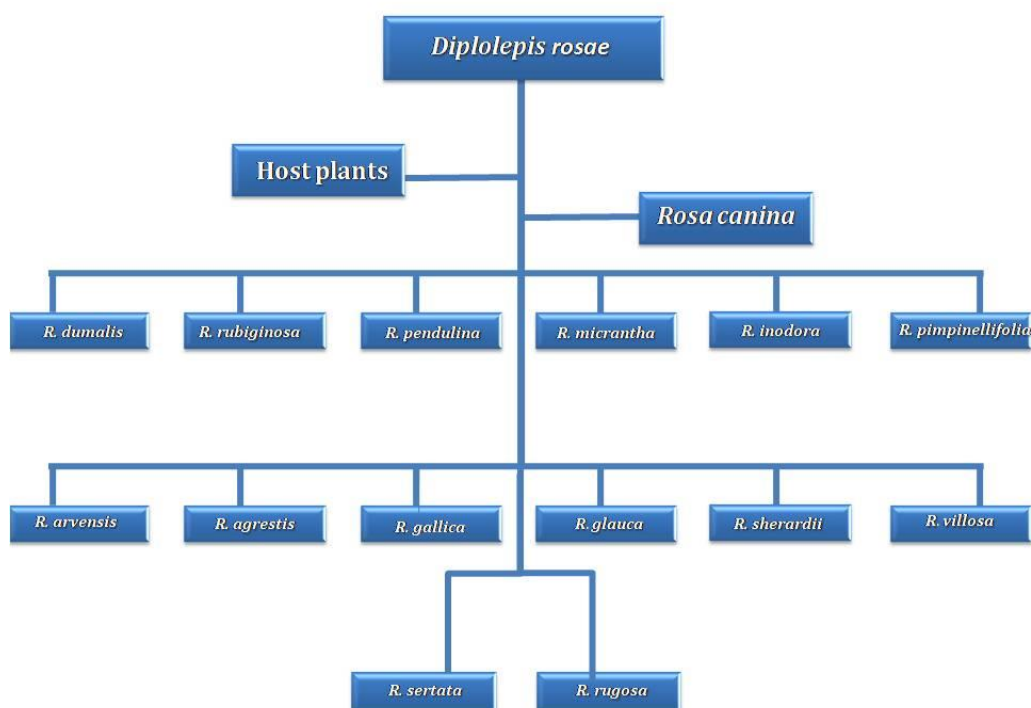


Fig. 1 - Host plant species for *Diplolepis rosae* (scheme based on information from the literature)

Plants of the genus *Rosa* respond to the attack of the species *Diplolepis rosae* by the appearance of hypertrophies and hyperplasia of procambial cells, which lead to the formation of galls. This type of damage (galls) is caused by the substances secreted by the larvae, and into a small share also contributes the feeding apparatus, causing mechanical injury to the cells. (URBAN, 2018).

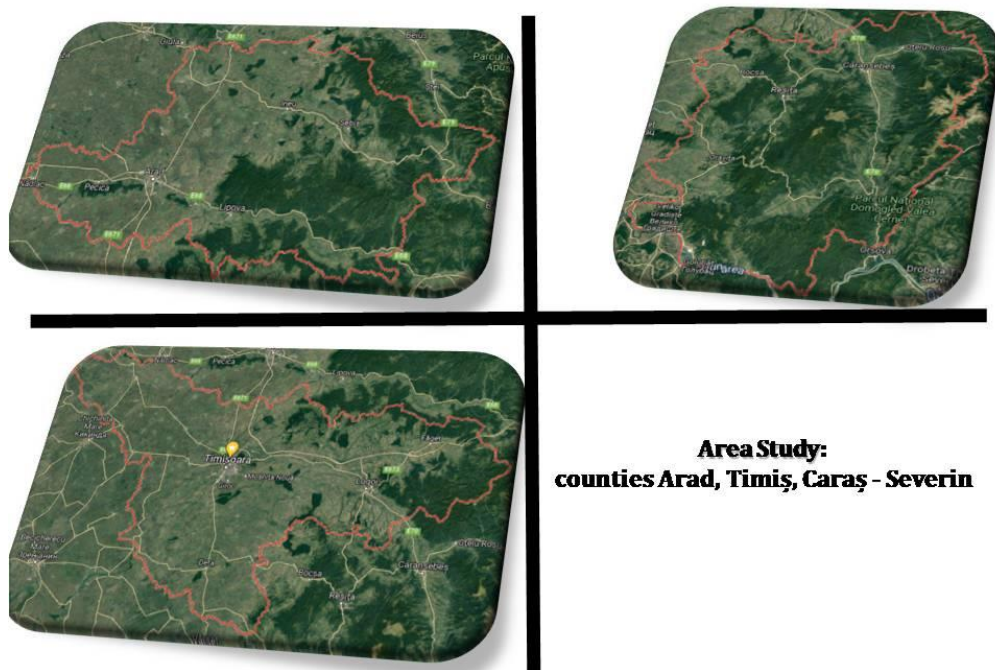
The larvae develop itself inside the galls, which provide them some protection against climatic conditions, predators and parasites. Galas are rich in protein and carbohydrates (<https://hortnews.extension.iastate.edu/authors/44>). These galls are shelters and food sources

for *Diplolepis rosae* larvae, ensuring their growth and development. New adults leave the galls next spring (<https://hortnews.extension.iastate.edu/authors/44>).

The purpose of this study is to report the species *Diplolepis rosae* in the western part of Romania and to describe the damage caused.

MATERIAL AND METHODS

The areas where the research was conducted are scattered in the western part of Romania. Between June 2019 and April 2021, surveys were conducted on the distribution area of the species *Diplolepis rosae*, in the counties of Timiș, Arad and Caraș-Severin. The sites where the observations were performed: Timiș [E21.336798°/N45.798092°; E20.978848°/N45.740765°; E21.314634°/N45.522912°; E21.308431°/N45.531691°; E21.314709°/N45.522922°; E21.106372°/46.000515°]; Arad [E21.437494°/N46.117570°]; Caraș Severin [E21.699936°/N44.888687°].



The sites studied were located in public areas. The data were collected from degraded pastures, from a pastoral point of view, invaded by *Rosa canina*. Galls produced by mossy rose gall wasp from rosehip plants were collected (October 2020 - April 2021) and transported to the laboratory to determine the mode of damage. In order to obtain an overview, regarding the galls produced by *Diplolepis rosae*, the diameter, weight and number of lodges/galls were determined. The digital caliper was used to determine the diameter of the galls. The weighing of the galls was performed with the Kern balance. To determine the number of lodges/galls, the galls were sectioned under the binocular magnifier.



RESULTS AND DISCUSSIONS

In Romania, the species *Diplolepis rosae* was reported in: Transylvania 46°47'N and 23°38'E, 46°31'N and 24°34'E (LÁSZLÓ, Z. and TÓTHMÉRÉSZ, B., 2008), Cluj County, Târgu Mureş (LUREZLÓ, Z. and TÓTHMÉRÉSZ, B., 2011); Bucharest (CHIRECEANU C. et al., 2015); Tinca Bihor county (ILIE A. L., MARINESCU MARIANA, 2018). LÁZLÓ Z. and PRÁZSMÁ H. (2019) signals the presence in Romania also other species of the genus *Diplolepis*: *Diplolepis spinosissimae* (Cluj), *Diplolepis eglanteriae* (Cluj, Hrg hita), *Diplolepis nervosa* (Cluj, Satu Mare) (figure 2).

The area of the species *Diplolepis rosae* (Cynipidae) in the Western region of Romania is shown in table 1.

Table 1

Distribution of the species *Diplolepis rosae* (Cynipidae) in the western region of Romania

Observations site	County	Latitude	Longitude	No. chambers/sit	Host plant
Giarmata Vii	Timiș	45.798092°	21.336798°	25	<i>Rosa canina</i>
Beregsău Mic	Timiș	45.740765°	20.978848°	5	<i>Rosa canina</i>
Herneacova	Timiș	45.522912°	21.314634°	24	<i>Rosa canina</i>
Recaș	Timiș	45.531691°	21.308431°	12	<i>Rosa canina</i>
Salciua Noua	Timiș	45.522922°	21.314709°	50	<i>Rosa canina</i>
Mănăstur	Arad	46.000515°	21.106372°	7	<i>Rosa canina</i>
Frumușeni	Arad	46.117570°	21.437494°	3	<i>Rosa canina</i>
Sasca Montană	Caras-Severin	44.888687°	21.699936°	17	<i>Rosa canina</i>
Total				143	

The data collected from the field indicate the presence of the species mossy rose gall wasp in seven sites in Timiș County, 1 site in Arad County and 1 site in Caraș-Severin County. In all the sites studied, the species *Diplolepis rosae* had as host plant the species *Rosae canina*. The number of galls produced by mossy rose gall wasp, in the nine analyzed sites, fluctuated between 3 - 50. In the site E21.314709°/N45.522922° the highest number of galas was registered.

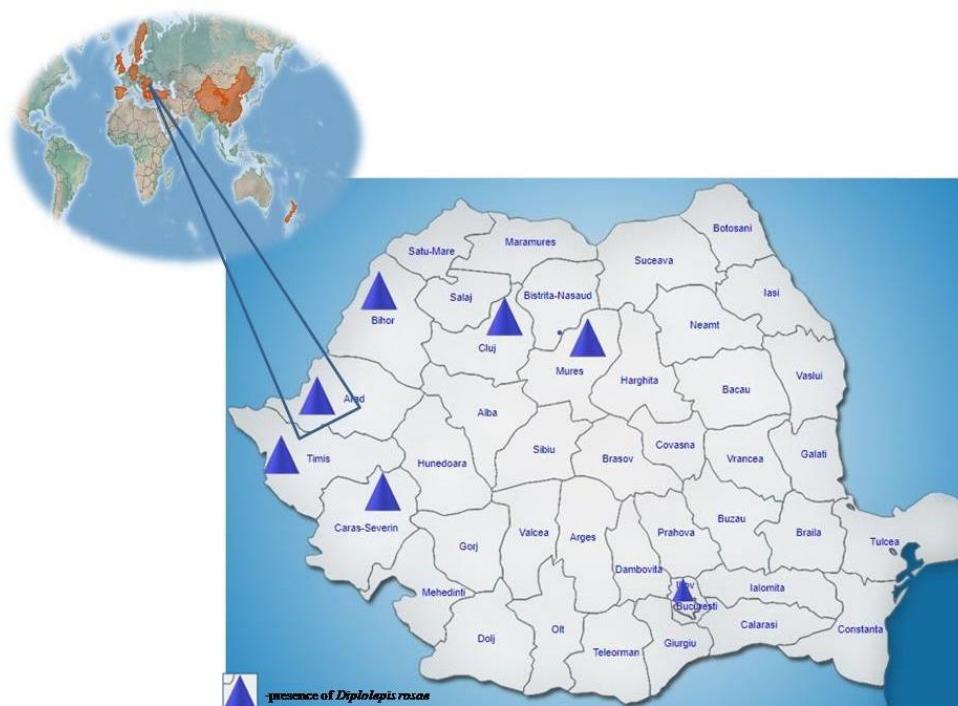


Fig. 2. - Presence of *Diplolepis rosae* in Romania

The sites where the species was present, *Diplolepis rosae*, were characterized by average annual temperatures of 10.0°C - 12.39°C (minimum of 10.0°C was recorded in the area Sasca Montană/Caraș - Severin, and maximum 12.39°C Frumușeni/Arad) and average annual rainfall of 459.3 mm (Frumușeni/Arad) - 900 mm (Sasca Montană/Caraș - Severin). The observation points in Timiș County were in the middle of the range (average temperatures of 10.6 - 10.9°C with rainfall of 600 - 631 mm) (GROZEA et al., 2009). The observations made by GUTIÉRREZ ET AL. (2021) in the Iberian Peninsula, confirms the presence of the pest at an average annual temperature of about 11°C and annual rainfall of 700–800 mm. *D. rosae* is present in the North - East (NE) and North - West (NW) regions of the Iberian Peninsula, where the annual temperatures average is 4°C - 19°C and rainfall 160 - 190 mm (GUTIÉRREZ ET AL. (2021). These data indicate the presence of the species *Diplolepis rosae*, in the western part of Romania, could be used together with previous publications. (LÁSZLÓ and TÓTHMÉRÉSZ, 2008; LÁSZLÓ and TÓTHMÉRÉSZ, 2011; CHIRECEANU ET AL., 2015; ILIE, MARINESCU MARIANA, 2018) in biogeographic studies of Romania.

The presence of mossy rose gall wasp depends on the host plant (*Rosa canina*), being a limiting factor of the distribution (NIEVES-ALDREY, 2001a; STILLE, 1984; KOHNEN ET AL.,

2011) along with climatic and geographical conditions. Studies on the influence of landscape diversity on the abundance of the *Diplolepis rosae* population are contradictory (unclear), LOONEY and EIGENBRODE (2010) claim that it has no effect or has low effect, LÁSZLÓ ET AL. (2018) states that the homogeneity of the landscape provides a perfect habitat.

After collecting the galls from *Rosa canina* species and transporting them to the laboratory, the weight, diameter and number of chambers/galls were determined.

The galls produced by *D. rosae* have a complicated structure, and their development depends on the number of eggs and larvae, the latter ones secreting a chemical substance that affects the plant tissue along with the mechanical action manifested through the feeding apparatus (MANI 1964, quoted by STILLE, 1984). The information presented by URBAN (2018) states that tissue proliferation is largely caused by young (newly hatched) larvae due to the irritating substances it secretes.

After collecting the galls from *Rosa canina* species and transporting them to the laboratory, the weight, diameter and number of chambers/gall were determined (figure 3).

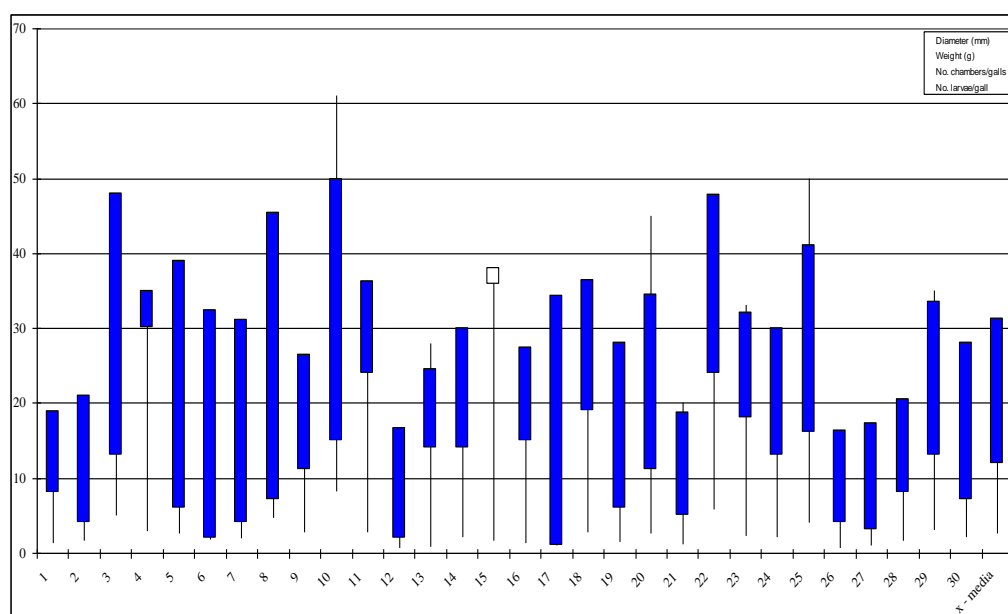


Fig. 3 - Results regarding the study of the galls produced by *Diplolepis rosae* plant of *Rosa canina* and the number of larvae present/galls

The collected galls presented variable dimensions, the smallest ones presented a diameter of 16.32 mm and a weight of 0.59 g. The largest galls collected had 50.01 mm and 8.13 g. The average value of the diameter was 31.26 mm, this value being also mentioned in up-to-date literature (URBAN, 2018). Galls with a diameter of less than 20 mm were 15%, 18% recorded values between 20 - 30 mm, 39% recorded values between 30 and 40 mm and only 18% recorded dimensions of 40 - 50 mm (figure 3). The average value of the analyzed galls diameter was 31.26 mm, which is higher compared to the averages recorded by LASZLÓ ET AL. (2014) 1.17 mm in 2008, 1.75 mm in 2009 (observations were made in Cluj County - Romania), respectively 2.30 mm in 2009 (Hungary).

After weighing the galls, it turned out that 76.67% had a weight of ≤ 3 g, 20% had values between 3-6 g and only 3.33% had a weight between 6 - 9 g, the amplitude of the results being high 7.54. The weight of the galls was not directly proportional to the diameter, one of the explanations being that in some lodges/galls the species *Diplolepis rosae* was not present, do the fact that the galls were collected in April, a period that coincided with the emergence of new adults (figure 4).

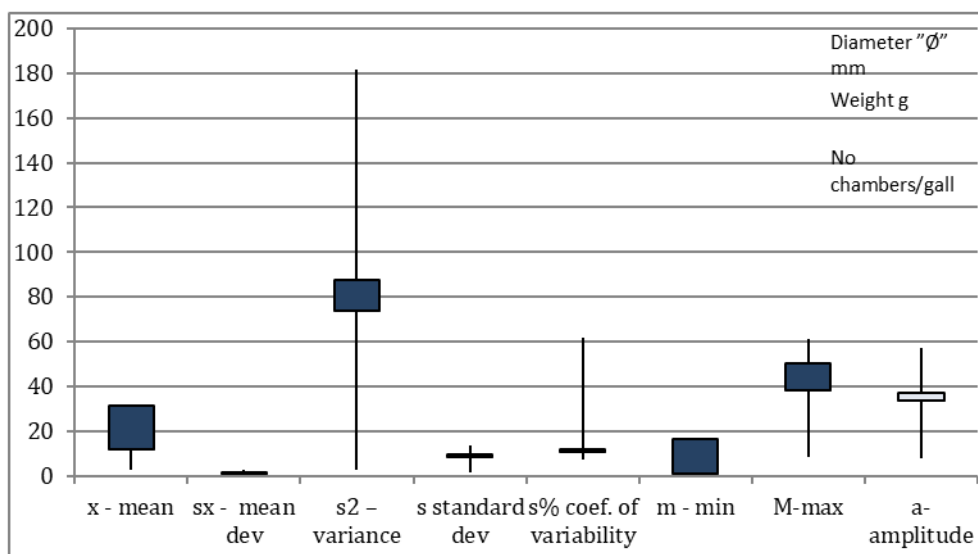


Fig. 4 - Statistical analysis of the results obtained on galls produced by *Diplolepis rosae* plants of *Rosa canina*

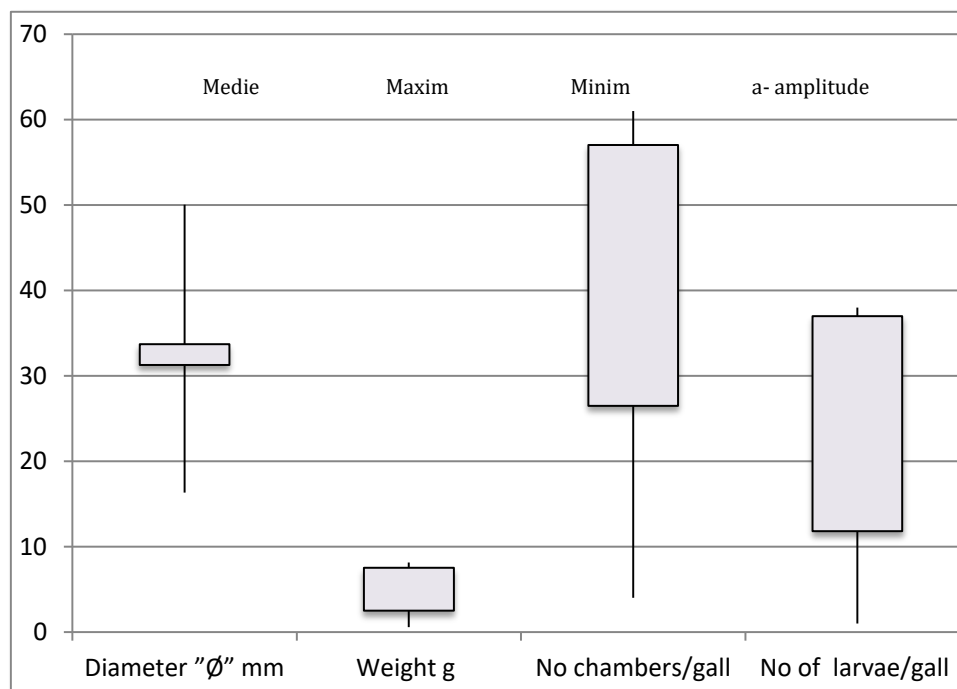


Fig. 5 - Results on the larval population of *Diplolepis rosae* and galls produced

After cutting a number of 30 galls, we counted a total of 794 lodges (chambers), the average/galls being 26.47. URBAN (2018), along with other researchers (SHORTHOUSE, 1998; SLIVA AND SHORTHOUSE, 2006), mentions that *Diplolepis rosae* produces multicameral galls, unlike other species of the genus *Diplolepis* that produce unicameral galls, a result confirmed by the present study. Following the sectioning of the galls, 4 to 61 lodges/gall were observed. From the point of view of the lodges/gall we can say that there is homogeneity, the coefficient of variability being 7.43 (figure 5).

The galls presented oval and round lodges (figure 6). The analyzed chambers were brown and blackish brown. Inside, the lodges have layers, distinct from the tissues, arranged concentrically (URBAN, 2018). The inner layer of these chambers contains nutrient cells, which are the only source of food for *Diplolepis rosae* larvae (URBAN, 2018).

The galls show on the surface (figure 6) muscle-like growths, they are soft and elastic at the beginning, then they become hard and brittle (they break). The young galls are green, and often red at the tips, reaching maturity they turn brown and then black. Following the study, it was observed that most small galls develop on young shoots and arranged laterally.

Larvae, pupae, adults were present in the analyzed lodges. The maximum number of larvae/galls was 38.



Fig. 6. - Development and life cycle of *Diplolepis rosae* (upper left - galls; upper middle – galls opened; upper right - larva; Middle left – pupae; middle right – before imago; Lower left – imago; lower right – adult)

The amplitude of the results regarding the number of larvae/galls (37) is very high, the results being not homogeneous, one of the explanations consist in pupae presence in the lodge, imago and adults (figure 6), a second one refers to the leaving of the lodge, the galls being harvested in April.

CONCLUSIONS

Diplolepis rosae is the most popular species in the genus *Diplolepis* due to its visible and abundant galls. The objective of the presented study was to examine the galls produced by the species *Diplolepis rosae* of rosehip plants and to signal the new sites where the species is present on the Romanian territory.

- The results of the study show that the species has expanded its areas in Romania, currently to be found in the counties of Timiș, Arad, Caraș-Severin, Cluj, Mureș, Bihor and in Bucharest.

- The weight of the galls is variable (the data being nonhomogeneous with large differences between minimum and maximum),

- The diameter of the galls and the number of lodges/galls are homogeneous.
- The number of larvae determined in a gala ranged from 1 to 38.

However, the species *Diplolepis rosae* has been reported in Romania since 2009, the faunal studies on cynipids are relatively few and at very beginning, which led to the lack of mapping of the species mossy rose gall. Thus, we believe that studies on monitoring, host plants and damages should continue.

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