# Mortality of *Metopolophium dirhodum* (*Homoptera*: *Aphididae*) Caused by Strains of *Erynia neoaphidis* (*Entomophthorales*: *Entomophthoraceae*) from Slovakia and Greece

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**Abstract**

Eliašová M., Papierok B., Cagáň Ľ. (2004): **Mortality of** *Metopolophium dirhodum* (*Homoptera*: *Aphididae*) **caused by strains of** *Erynia neoaphidis* (*Entomophthorales: Entomophthoraceae*) **from Slovakia and Greece.** Plant Protect. Sci., **40**: 54–62.

The aim of the study was to test isolates of *Erynia neoaphidis* originating from Slovakia and Greece for infectivity to *Metopolophium dirhodum*, and evaluate selected strains for their infectivity at different temperatures. For alatae, an $LC_{50}$ (number of spores/mm$^2$) from 109.97 to 230.90 was calculated for the Slovakian strain and of 218.9 for the Greek strain. For apterae, the $LC_{50}$ was 82.2–194.5 for the Slovakian strain and 367.2 for the Greek strain. The temperature during the incubation period influenced mortality and length of life cycle of the fungus. Lower temperature decreased the mortality and extended the incubation time. With the Slovakian strain and at 22°C, the highest proportion of individuals killed by the fungus was recorded on the third day of incubation (47.8% of the alatae and 49.9% of the apterae), while at 11°C most individuals died on the seventh day (55% and 37.5% of alatae or apterae, respectively). With the Greek strain and at 22°C, the highest proportion of individuals killed was recorded on the fourth day of incubation (40.6% of alatae and 57.9% of apterae). A lower incubation temperature (11°C) prolonged the life cycle and most apterae killed by this strain were recorded on the ninth day (35%), while the highest proportion of killed alatae was recorded on the tenth day (42.8%).

**Keywords:** *Erynia neoaphidis; Metopolophium dirhodum; Entomophthorales*; Slovakia; Greece

Development of resistance to certain insecticides in aphid populations highlights the importance of biological control as a tool for pest management (Wells *et al*. 2000). The most common and obvious aphid diseases are fungal species of the order *Entomophthorales*. Fungal entomopathogens such as *Beauveria bassiana* (Balsamo) Vuillemin or *Paecilomyces* spp. showed a promising level of activity against aphids, but they can also kill aphid predators such as coccinellids (Milner 1997; Cagáň & Uhlík 1999). The fungus *Erynia neoaphidis* Remaudière & Hennebert is widespread in nature and frequently cited as the species most often infecting aphids (Thoizon 1970; Remaudière *et al*. 1978, 1981; Miętkiewski & Van der Geest 1985; Bałazy *et al*. 1990; Stalmachová & Cagáň 2000). It is often referred to as the dominant fungus infecting cereal aphids (Coremans-Pelseneer 1976; Dedryver 1981, 1983; Papierok & Havukkala 1986; Feng & Johnson 1991) and is one of the species that are easily cultured *in vitro* (Latgé *et al*. 1983). Dedryver (1981) preferred this species for use as biological control agent because of its quite large host spectrum and excellent aggressivity.

Observations of natural infection in the field suggest that the fungi in the initial stages of epizootics spread too slowly to prevent aphid damage. A higher initial infection level after ap-

plication should provide better control (Wilding 1981; Wilding *et al*. 1986, 1990). An analysis of 148 cases of experimental or field introduction of an entomopathogen as biocontrol agent found that the selection of the wrong strain was one of the most important reasons for failure (Wysoki 1998). The infectivity of a pathogen represents the ability to attack a host and cause the infection, and it is estimated by the value $LD_{50}$ (Papierok 1982).

The aim of this study was to isolate *E. neoaphidis* strains from infected aphids originating from different climatic conditions of Europe (France, Slovakia, Finland and Greece), test the isolates for infectivity to *Metopolophium dirhodum* (Walker), and evaluate selected strains for their infectivity in conditions with different temperature.

## MATERIALS AND METHODS

***Isolation of** Erynia neoaphidis **strains.*** The following medium was used for isolation and maintaining fungi belonging to the order *Entomophthorales*: 18 g Sabouraud dextrose agar, 4 g agar, 4 g glucose, 2 g yeast extract and 600 ml distilled water were put into a large Erlenmeyer flask. This was then sterilised for 30 min at 114°C, together with other material needed: 100 ml of whole milk, forceps, eprouvette of 250 ml and glass cane. Six eggs were superficially sterilised in a mixture of 98% alcohol and 10% sodium hypochlorite for 10 min. The yolks were removed after breaking the eggshells with sterile forceps (near a Bunsen burner) and poured into a sterile eprouvette. Sterile milk was blended with the egg yolks using a sterile glass cane. The mixture was added to the sterile Sabouraud dextrose agar (not very hot) and mixed by careful shaking. The medium was then ready for distribution into tubes or Petri dishes.

To isolate the pathogen, an infected aphid was placed on a piece of damp cellulose inside a small Petri dish lid (ø 30 mm). The lid was placed over a bottom containing the medium. After approximately 12 h the bottom was removed, covered by a sterile lid, and another bottom with new medium placed under the lid with the infected aphid. This change of dishes was done several times and about every 2 h, depending on sporulation intensity. Then the dishes were kept at laboratory conditions (22 ± 1°C, 12/12 h light/dark regime).

After fungal growth, a piece of mycelium was placed into a tube with the same medium used for isolation. The cultures were kept at 11°C and 12/12 h light/dark regime.

***Aphid species and fungal strains used in the experiment***. The aphid species used in the experiments was *M. dirhodum,* maintained by the Department of Entomopathogenic Fungi and Bacteria, Pasteur Institute, Paris. Alatae and apterae females of the aphid were tested. The strains GR42 and sk(NR)1 of *E. neoaphidis* were chosen. They originated from different climatic conditions. The aphid species from which the strains were isolated, country of origin and year of isolation are given in Table 1.

Table 1. Strains of *Erynia neoaphidis* used in the experiment

| Name of the strain | Host aphid species | Country of origin | Year of isolation |
|---|---|---|---|
| GR42 | *Sitobion avenae* | Greece | 1997 |
| sk(NR)1 | *Metopolophium dirhodum* | Slovakia | 1998 |

***Evaluation of the strains for their infectivity in different conditions***. The experiment included preparation of the inoculum, infection, incubation and evaluation.

To prepare the inoculum the strains were grown in 60 mm Petri dishes on the medium described above. After the culture covered the medium surface, the mat of mycelium was lifted off the medium. The mat was cut into small pieces and placed on damp cellulose in a small Petri dish lid (ø 35mm). The lid was placed over a microscope slide. After 12 h the intensity of sporulation was checked on the slide.

To achieve infection, the aphids were inoculated in a small Petri dish (ø 35 mm). Aphids were placed in the bottom of the dish, and a sporulating mat was placed over it, with a piece of gauze between mat and aphids to prevent them from coming into direct contact with the fungal material. A small cover slip (14 × 14 mm) was placed in each Petri dish to capture conidia for later estimation of the spore concentration received by the aphids. To provide a range of doses, four to five different inoculation times were used, in most cases 5, 10, 30 and 60 min. Six replicates were made for each inoculation time. Each replicate contained 10 aphids of a given flight morph.

After exposure to the pathogen, the aphids were transferred for 24 h into a plastic cylinder (height 75 mm, Ø 50 mm). Two pieces of damp filter paper (40 × 20 mm) placed on the internal wall of the cylinder ensured the saturated conditions appropriate for spore germination. As food for the aphids there were three pieces of wheat leaves inside the cylinder. Aphids that died during the 24 h were excluded from the final calculation of the infection rate; this mortality was with high, probably caused by injuries during manipulation.

After 24 h the aphids were placed in plastic boxes with young wheat plants growing on wet cellulose. If the aphids were incubated at 22°C, the mortality was recorded daily for one week. If the incubation temperature was 11°C, the mortality was recorded daily for two weeks.

At the low relative humidity in the laboratory the fungus did not sporulate but mummified the aphid body. All dead aphids were, therefore, placed on damp cellulose for at least 12 h to determine through presence or absence of sporulation whether the fungus had killed them.

Fifteen experiments were conducted to determine the $LC_{50}$ (Table 2). In each experiment the concentration of spores received by the aphids and their mortality in each repetition were estimated, e.g. in the experiment on November 1998 infection times were 15, 30, 45 and 60 min, each with five concentration of spores used for 10 aphids – together 20 concentrations of spores and 200 aphids were used in the experiment. (For each concentration of spores the level of aphid mortality was determined.) Spore concentrations and mortality data were submitted to probit analysis using the corresponding procedure in SAS software to calculate $LC_{50}$ (the concentration of spores required to kill 50% of the aphids).

## RESULTS

### Evaluation of two fungal strains for their infectivity – the level of $LC_{50}$

Fifteen experiments were conducted. The value $LC_{50}$ was calculated only for eight of them; weak dependence of mortality on spore concentration did not allow stating the $LC_{50}$ for the other seven experiments. This happened particularly with strain GR42. The stated values are given in Table 2.

### Influence of temperature during incubation on the mortality of aphids

The temperature during the incubation period influenced mortality and length of time for the fungus to develop. Lower temperature (11°C) during

Table 2. Values of $LC_{50}$ (number of spores/mm$^2$) for *Erynia neoaphidis*, strains sk(NR)1 and GR42, during several dates of laboratory experiments in 1998 (incubation temperature 22°C)

| Strain | Date of experiment | $LC_{50}$ for alatae | $LC_{50}$ for apterae |
|---|---|---|---|
| sk(NR)1 | November 5 | 158.23 | * |
| | November 16 | * | 149.34 |
| | November 29 | 109.97 | * |
| | December 8 | not stated | 82.2 |
| | December 29 | 230.9 | 194.5 |
| | January 18 | not stated | not stated |
| | January 26 | * | not stated |
| GR42 | November 5 | * | not stated |
| | November 16 | not stated | * |
| | November 29 | not stated | * |
| | December 29 | 218.9 | 367.2 |

*the experiment was not made on the date given;
not stated – the variability of mortality in the experiment was too high to reliably calculate $LC_{50}$

Table 3. Mortality of *Metopolophium dirhodum* infected by *Erynia neoaphidis* strains sk(NR)1 and GR42, depending on the spore concentration, temperature during incubation and aphid form (at 11°C the mortality of aphids was recorded after 12 d, at 22°C after 7 d)

| Strain | Temperature during incubation (°C) | Alatae | | Apterae | |
|---|---|---|---|---|---|
| | | concentration of spores (number/mm$^2$) | mortality (%) | concentration of spores (number/mm$^2$) | mortality (%) |
| sk(NR)1 | 11 | 176.5 | 35.2a | 156.5 | 26.7a |
| | 22 | 153 | 52.2b | 138.6 | 58.0c |
| | | 156.3 | 65.5b | 150.8 | 66.7c |
| | | 134.3 | 41.3ab | 196.8 | 60.4c |
| | | 194.0 | 42ab | 137.8 | 30.0a |
| | | 102.8 | 38.5a | 73.8 | 41.7b |
| GR42 | 119 | 130.75 | 18.0a | 179.2 | 33.3ab |
| | 22 | 149.6 | 16.0a | 230.6 | 38.6b |
| | | 90.5 | 37.5b | 158.8 | 60c |
| | | 192.3 | 23.8b | 196.7 | 27.7a |
| | | – | – | 182.0 | 52.2c |

Means marked with the same letter are not significantly different ($P$ = 0.05, Tuckey multiple range test)

incubation decreased the mortality but increased the time needed for incubation.

In Table 3 the mortality depending on the concentration of spores and temperature during incubation is compared. For incubation temperature 22°C those values of spore concentration were selected that were closest to the spore concentrations used in the experiment with incubation temperature 11°C. In both strains and aphid forms, lower incubation temperature usually led to lower mortality.

### Aphid mortality during the incubation period

***Strain sk(NR)1***. If the aphids were incubated at 22°C, in two cases the first alatae individuals killed by this strain were found during the first day of incubation. The first dead apterae were recorded on the second day. The highest proportion of individuals killed by the fungus was recorded on the third day of incubation (47.8% of the alatae and 49.9% of the apterae). If the temperature of incubation was 11°C, the first apterae and alatae females killed by the fungus were found on the fourth and fifth day, respectively. Most individuals died on the seventh day of incubation, 55% of the alatae and 37.5% of the apterae. The last infected and killed individuals were recorded on the tenth day of incubation (Figure 1).

***Strain GR42***. At an incubation temperature of 22°C, the first apterae killed by this strain were recorded during the first day of incubation; with alatae it was the third day. The highest proportion of individuals killed by the fungus was recorded on the fourth day of incubation (40.6% of the alatae and 57.9% of the apterae). At a lower temperature (11°C) during incubation the first dead apterae were found on the seventh day of incubation, and the highest proportion killed by this strain were recorded on the ninth day (35%). For alatae the situation was slightly different; the first dead individuals were observed on the ninth day and the highest proportion of them was recorded on the tenth day (42.8%) (Figure 1).

## DISCUSSION

Table 2 shows that the values of $LC_{50}$ were different on each day of the experiment. The large confidence limits of each experiment did not al-

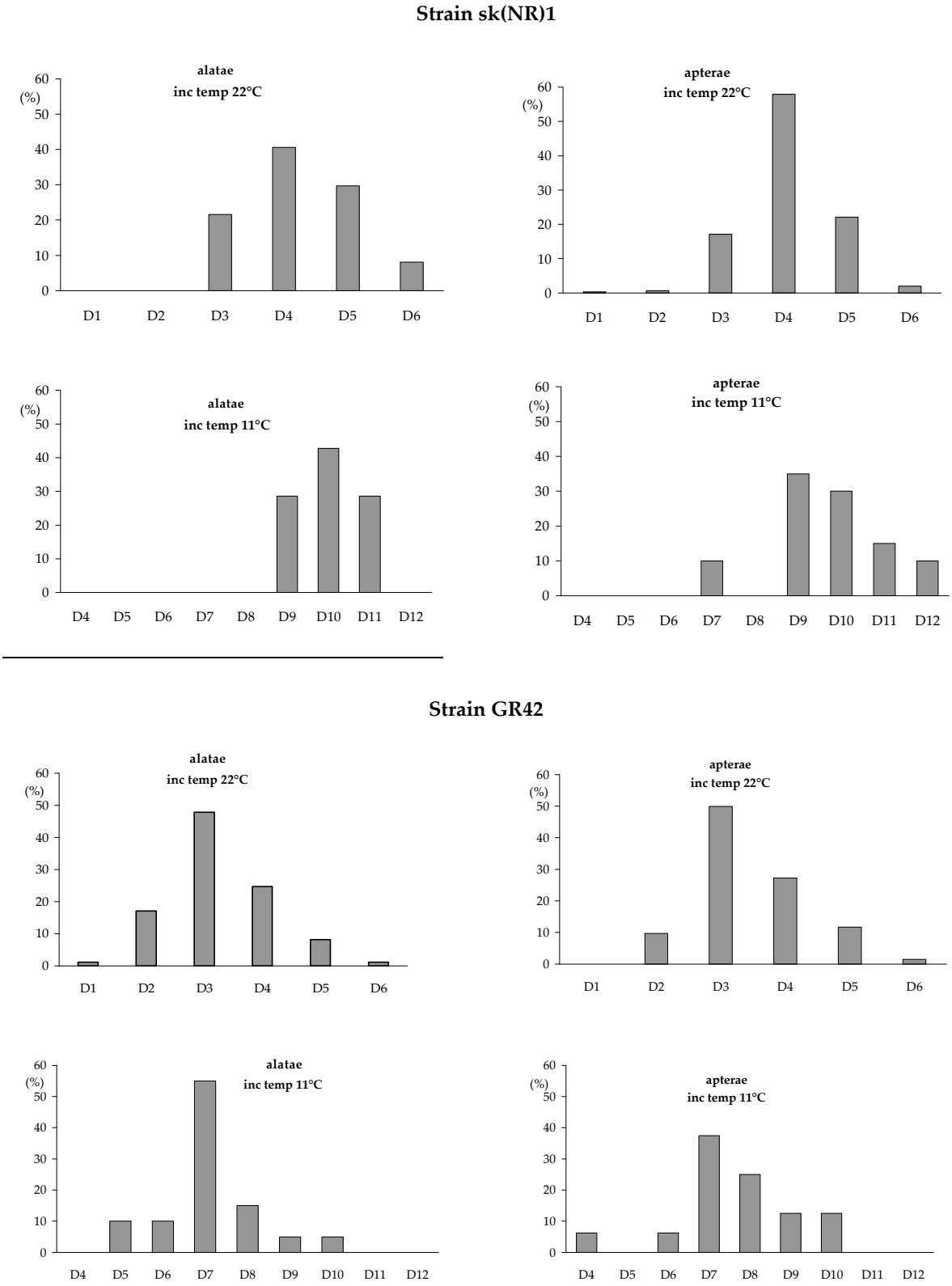


Figure 1. Mortality of *Metopolophium dirhodum* individuals during successive days (D1–D12) after exposure to primary spores of *Erynia neoaphidis*, strains sk(NR)1 and GR42. The total of aphids dead at a given date after exposure in all experiments (characterised by fungal strain, aphid morph and incubation temperature) was used to calculate frequency (%)

Table 5. Values of $LC_{50}$ (number of spores/mm$^2$) stated for *Erynia neoaphidis* strains used in experiments of several authors

| Reference | $LC_{50}$ | Aphid species used in experiment |
|---|---|---|
| Lizen *et al.* (1985)* | 0.31–2.64 | *Acyrtosiphon pisum* |
| Sierotzki *et al.* (2000) | 16 | *Acyrtosiphon pisum* |
| Sivčev & Draganić (1994) | 6.47 | *Brevicoryne brassicae* |
| Feng & Johnson (1991) | 4.3–7.2 | *Diuraphis noxia* |
| | 0.8–3.0 | *Metopolophium dirhodum* |
| Dromph *et al.* (2002) | 0.8–3.6 | *Sitobion avenae* |

*only the values of $LC_{50}$ for adults are given

low to make statistical comparisons of the results. The values of $LC_{50}$ found in our experiments were far higher than those reported by other workers (Table 4).

It is likely that the strains used in the experiments listed on Table 3 were more virulent than the two strains used in our experiments. However, some differences in procedure could also have contributed to some extent to the higher values of $LC_{50}$ reached in our experiments. Feng and Johnson (1991) as well as Lizen *et al.* (1985) used aphids of exactly defined age. The former authors used nymphs that were not older than 4 d, while the latter used 2–3 d old females. In our experiments the age of aphids used was quite variable, from freshly moulted to older individuals. Lizen *et al.* (1985) observed that aphids younger than 24 h after moulting are more susceptible to infection than older aphids. On the other hand, the variability in age of aphids used in our experiments reflects more the age variability occurring in nature.

There were also differences in methods used in the experiments. Feng and Johnson (1991) and Lizen *et al.* (1985) had by $CO_2$ narcotised and thus immobilised the aphids during inoculation time. Our aphids moved freely during inoculation time which, without doubt, increased the variation in actual doses received. This led to a poor correlation between the spore doses used and mortality.

Several authors have tested the influence of temperature on the infectivity and length of incubation period of Entomophthoralean strains. The incubation period of Entomophthorales and also other insect pathogens is clearly important in field epizootics. The incubation period of *E. neoaphidis* is strongly influenced by temperature (Wilding 1977). When moisture is not limiting, temperature over the range 10–20°C had no consistent effect on the infectivity of primary spores of *E. neoaphidis*. However, the time it took to kill an aphid increased as temperature decreased, from 3–5 d at 20°C to 12–15 d at 8°C (Milner & Bourne 1983). These data are comparable with our results. At 20°C infected aphids died after 3–6 d, while at 16°C and 6°C the corresponding period was 3–7 and 13–16 d (Sivčev & Draganić 1994). It took pea aphids inoculated with *E. neoaphidis* and incubated at 5°C about four times longer to die than those incubated at 25°C (Wilding 1977).

Our choice of strains for experimental infection was not random. We planned to test the influence of low temperature on strains originating from climatically different regions. The experiments confirmed our supposition that strains from climatically colder regions are less negatively influenced by low temperature. The distinctly longer incubation period needed by strain GR42 at 11°C could thus be explained by its origin.

In Greece, the temperatures during the main growing period are higher than those in Slovakia. The life cycle of an obligate pathogen depends on the life cycle of its host. Likewise, the evolution of the pathogen is also strongly linked to the evolution of its host. Optimal temperatures for the development of cereal aphids in Spain (Asin & Pons 1996) were higher than those for cereal aphids in colder countries (Carter *et al.* 1980; Michels & Behle 1989). We could thus suppose that the optimal temperature for growth of strains originating from Greece and for their spread in a host population is higher than that of strains from Slovakia or from other cold regions.

The influence of temperature on incubation period with respect to epizootic development seems important in Slovakia. Although the maximal day temperatures during the first two decades of June are suitable for fungus development, minimal day temperatures are in most cases below 15°C.

In our field survey, the first two phases of epizootic development (e.g. implantation of the pathogen and multiplication of infection centres) occurred during the first two decades of June. Usually, during the first half of June the mornings and evenings are relatively cold, and higher temperatures occur for a shorter time during daytime than at the end of June and in July. Thus, within 24 h the development of the fungus in its host occurs at mostly low temperatures. We suppose that the length of incubation influences the duration of those two phases, especially the second one. A short incubation time can shorten the time needed for multiplication of infection centres within the crop. Thereafter, an epizootic (third phase) can start sooner and hamper an aphid outbreak. The idea to use Entomophthorales in biological control of aphids is to support the natural occurrence of entomopathogens during the early stages of development of the host population (Wilding *et al.* 1990). In the climatic conditions of Slovakia it could be better to choose strains that do not react to low temperature by a marked extension of the incubation period. Wraight *et al.* (1993) also emphasised the selection of pathogen strains with below-normal temperature optima for the control of early season aphid populations.

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## Súhrn

Eliašová M., Papierok B., Cagáň Ľ. (2004): **Mortalita** *Metopolophium dirhodum* (*Homoptera: Aphididae*) **spôsobená izolátmi huby** *Erynia neoaphidis* (*Entomophthorales: Entomophthoraceae*) **pochádzajúcimi zo Slovenska a z Grécka**. Plant Protect. Sci., 40: 54–62

Testovali sme infekčnosť izolátov huby *Erynia neoaphidis* Remaudière & Hennebert (*Entomophthorales*: *Entomophthoraceae*) pochádzajúcich zo Slovenska a z Grécka vo vzťahu k voške *Metopolophium dirhodum* (Walker) (*Homoptera: Aphididae*) pri rôznych teplotách. V prípade okrídlených foriem bola vypočítaná hodnota $LC_{50}$ od 109,97 do 230,90 spór na mm$^2$ pri použití izolátu huby zo Slovenska a 218.9 spór na mm$^2$ pri použití izolátu huby z Grécka. Pre neokrídlené formy sa stanovila $LC_{50}$ 82,2–194,5 spór na mm$^2$ v prípade izolátu zo Slovenska a 367,2 spór na mm$^2$ v prípade gréckeho izolátu. Teplota počas inkubačného obdobia ovplyvňovala mortalitu vošiek aj rýchlosť vývinu huby. Nižšia teplota počas inkubácie (11 °C) znížila mortalitu a zvýšila dĺžku inkubačného obdobia. V prípade izolátu zo Slovenska sa zaznamenal pri teplote 22 °C najvyšší počet jedincov usmrtených hubou na tretí deň inkubácie (47,8 % alatae a 49,9 % apterae). Pri teplote inkubácie 11 °C väčšina jedincov zahynula na siedmy deň inkubácie (55 % alatae, resp. 37,5 % apterae). V prípade izolátu z Grécka bola pri teplote 22 °C najväčšia časť usmrtených vošiek zaznamenaná na štvrtý deň inkubácie (40,6 % alatae, resp.

57,9 % apterae). Nižšia teplota (11 °C) počas inkubácie predĺžila životný cyklus huby a väčšina neokrídlených jedincov bolo týmto izolátom usmrtených na deviaty deň (35 %). Najväčšia časť usmrtených okrídlených jedincov sa zistila na desiaty deň (42,8 %).

**Kľúčové slová**: *Erynia neoaphidis*; *Metopolophium dirhodum*; *Entomophthorales*; Slovensko; Grécko

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