JOURNAL OF FOREST SCIENCE, *52*, 2006 (3): 136–140

Infection of wintering pupae of horse-chestnut leafminer *Cameraria ohridella* Deschka et Dimić. by *Verticillium lecanii* (Zimmerman)Viégas

T. Samek[1], D. Novotný[2], L. Jankovský[3]

[1]*Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic*
[2]*Research Institute of Crop Production, Praha-Ruzyně, Czech Republic*
[3]*Faculty of Forestry and Wood Technology, Mendel University of Agriculture and Forestry Brno, Brno, Czech Republic*

ABSTRACT: The population dynamics of the horse-chestnut leafminer *Cameraria ohridella* Deschka et Dimić. was investigated in 1997–2000. The mortality of wintering pupae and the rate of fungal infections were also examined. The mortality of the horse-chestnut leafminer was nearly 40% at the end of spring. The spectrum of fungi on examined dead pupae was as follows: *Verticillium lecanii* (Zimmerman) Viégas, *Cladosporium herbarum* (Persoon: Fries) Link, *Cladosporium cladosporioides* (Fresen.) de Vries, *Alternaria alternata* (Fr.) Keissler, *Acremonium strictum* W. Gams, *Rhizopus stolonifer* (Ehrenberg: Fries) Lind., *Arthrinium phaeospermum* (Corda) Ellis and *Acremonium* sp. 1. *Verticillium lecanii* was the only identified fungus with an entomophagous status. The fungus is, however, a well documented entomopathogen of *Homoptera*, particularly of aphids, scale insects and whiteflies in tropical and subtropical regions.

Keywords: *Cameraria ohridella*; horse-chestnut leafminer; horse-chestnut; entomophagous fungi; *Verticillium lecanii*; mortality; population dynamics

Entomopathogenic fungi are an important mortality factor participating in the maintenance of the insect population balance. A number of entomopathogenic fungi has already been used as a biological agent in insect pest control in agriculture and forestry. The best known are members of the genus *Beauveria,* particularly strains of the species *Beauveria bassiana* (Bals) Voill attacking, among others, beetles and butterflies. In the forest pest control using biological means suspensions of spores under trade names Boverol or Boverosil are applied. The spectrum of entomopathogenic fungi is, however, substantially broader similarly like the spectrum of offered biorational insecticides. Effects of entomopathogenic fungi on wintering pupae of *Cameraria ohridella* Deschka et Dimić can be considered to be potential means how to reduce the present high population density of the pest.

MATERIAL AND METHODS

Sampling

Material from the Brno-Modřice locality was sampled starting in 1998. Leaf litter collected in early spring served as the basic material for laboratory studies. A certain number of wintering pupae which were then the subject of detailed investigations was obtained from the litter.

The first examination was carried out after the winter 1997/1998. After the swarming of imagoes of the wintering generation finished, two samples of litter were taken: (1) from a photoeclector which served for the observation of the course of swarming and (2) from a place where the litter was sampled. Subsequently, after opening created "mines", mortality of wintering pupae of *Cameraria ohridella* or

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. 6215648902.

Table 1. Mortality of pupae of a wintering generation, Modřice locality, winter 1997–1998

Sampled from	Mines left by *Cameraria ohridella* (%)	Mines left by chalcids (%)	Attacked by *Cameraria ohridella* (%)	Attacked by chalcids (%)
(1) photoeclector	62	0	38	0
(2) open area	65	6	27	2

Table 2. Development of the mortality of pupae of a wintering generation, Modřice locality, winter 1998–1999

Date	Percentage of pupae		Mean monthly air temperature (°C)
	attacked	healthy	
27 December	11	89	–1.9
27 January	14	86	–0.8
27 February	14	86	0.1
27 March	33	67	6.4
10 April	35	65	11.5

Values of the air temperature are taken from a climatological station in Žabčice

parasitoid chalcids (*Chalcidoidea*) was identified by an ocular estimate.

During the winter period 1998/1999, the study was repeated. Material was sampled at the same place of the locality. In the interval of a month, samples were continuously subject to the ocular examination of the rate of pupae attack. Within the study of the mortality of pupae, 20 pupae attacked by fungi were sampled in April 1999. The pupae were used for the isolation and determination of fungi.

After the swarming of imagoes of the wintering generation 1999/2000 finished, 20 dead pupae showing no visible symptoms of attack and 4 pupae evidently covered with mycelium were obtained from the photoeclector. This amount of pupae was divided into two groups and within the groups (1) fungi on the surface of pupae and (2) fungi inside the pupae were studied.

Fungi isolation

(1) **Fungi from the surface of pupae.** Pupae were shaken in an ultrasonic bath in sterile water with the admixture of a soaking agent for a period of 1 minute. Isolation was carried out using the method of dilution. We studied a sample of 4 pupae obviously covered with mycelium and of 5 pupae without the evident presence of surface mycelium.

(2) **Fungi inside the pupae** were studied on the sample of 15 pupae with the visible presence of mycelium on their surface. In the whole group of pupae, surface sterilization was carried out using spirit. Sterilized pupae were singly placed on an agar/malt plate. Fungi were cultivated on 3% malt extract agar at a laboratory temperature of 23°C.

RESULTS

The mortality of *Cameraria ohridella* pupae was almost 40% at the end of the wintering period (Tables 1 and 2). Differences in the rate of attack between samples from the photoeclector and from the open area found in spring 1998 were obviously a result of the sporadic and uneven occurrence of chalcids (Table 1). Of course, these parasitoids were also attacked by fungi. Mortality in spring 1998 and 1999 was comparable (Tables 1 and 2). However, the mortality of pupae was significantly affected by the course of the winter period.

In 1999, the following spectrum of fungi was identified in the sample of 20 pupae: *Verticillium lecanii* (Zimmerman) Viégas, *Cladosporium herbarum* (Persoon: Fries) Link, *Cladosporium cladosporioides* (Fresen.) de Vries, *Alternaria alternata* (Fr.) Keissler, *Acremonium strictum* W. Gams, *Rhizopus stolonifer* (Ehrenberg: Fries) Lind.

Verticillium lecanii was the most frequently isolated species forming cylindrical conidia of white colonies in awl-shaped phialides. According to Gams (1971), it refers to an aggregate of tiny species. Just *V. lecanii* is the only isolated species mentioned as an entomophagous fungus.

In 2000 six species of fungi were isolated on pupae. The following species were determined on the surface of pupae: *Alternaria alternata*, *Verticillium* sp. 1, *Verticillium* sp. 2, brown sterile mycelium; from the surface-sterilized pupae *Alternaria alternata*, *Arthrinium phaeospermum* (Corda) Ellis, *Acremonium* sp. 1, brown sterile mycelium. These species are rather saprophytic ones and none of them is a typical entomophagous species. The spectrum of the

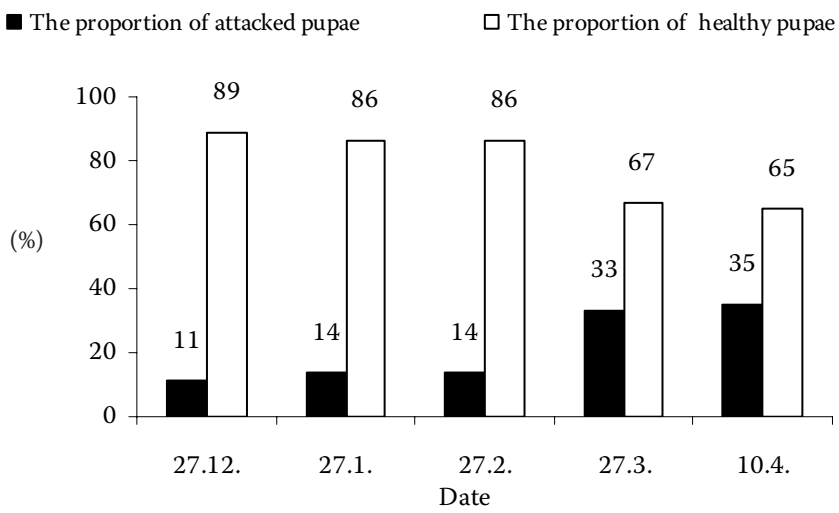

Fig. 1. Development of the mortality of pupae of a wintering generation, Modřice locality, winter 1998–1999

studied fungi on wintering pupae coming from both years was markedly different. The only species found in both years was widely distributed *Alternaria alternata*. The occurrence of the genus *Verticillium* was also detected in both years. *Verticillium lecanii*, very abundant in 1999, was not found in 2000.

DISCUSSION

Mortality of wintering pupae has not been published in scientific literature dealing with *Cameraria ohridella* yet (with the exception of the paper of Mráz 1999). Nevertheless, the effects of entomopathogenic fungi on the population density of the species are obvious and much higher than e.g. the effects of traditional enemies of mining species from the order of *Hymenoptera*.

For example, the proportion of parasitism on the part of chalcids was repeatedly corroborated in the order of several percents. However, there arises a question if native species of the parasites are able to adapt themselves to a new host. If they were not, it would be necessary to look for other ways of reducing the present high population density of the pest because the present condition is not obviously permanently possible. One of the possibilities is to use entomopathogenic fungi. Particularly the use of species parasitizing on pupae can be taken into consideration. *Verticillium lecanii* was the only entomophagous species in the studied sample determined yet.

Verticillium lecanii is a relatively well examined entomopathogenic fungus on *Homoptera*, in the majority of aphids, scale insects and whiteflies particularly in tropical and subtropical regions. *V. lecanii* occasionally hyperparasitizes on phytopathogenic fungi, mostly rusts and mildew (Hall 1981). However, it has not been noted in pupae of butterflies yet. The virulence of *V. lecanii* is associated with the production of extracellular chitinases. As for toxins, the production of cyclodepsipeptides was detected. In this country, Khalil et al. (1983) dealt with cul-

Table 3. A survey of the determined species of fungi on wintering pupae of *Cameraria ohridella*

Species	Number of infected pupae	
	1999	2000
Verticillium lecanii (Zimmerman) Viégas	8	0
Cladosporium herbarum (Persoon: Fries) Link	3	0
Rhizopus stolonifer (Ehrenberg: Fries) Lind.	2	0
Alternaria alternata (Fr.) Keissler	3	3
Acremonium strictum W. Gams	1	0
Cladosporium cladosporioides W. Gams	1	0
Arthrinium phaeospermum (Corda) Ellis	0	1
Acremonium sp.	0	1
Verticillium sp. 1	0	2
Verticillium sp. 2	0	1
Brown sterile mycelium	0	2

tivation conditions for *V. lecanii*. A preparation with the concentration of spores 5×10^7–1×10^9 per ml with the addition of a soaking agent is mostly used for aphid control. *Verticillium lecanii* is used for the control of *Coccus viridis* in tropical regions, particularly in Cuba and Florida. In greenhouse cultures, *V. lecanii* is used for *Bemisia tabaci* control. On the basis of *V. lecanii*, some other preparations are manufactured designated for the control of aphids (Vertalec), scale insects, and whiteflies (Mycotal, Savona). From the viewpoint of potential parasitization on pupae of *Cameraria ohridella*, however, it will be necessary to carry out a number of other tests and observations.

CONCLUSION

On the basis of studies carried out in the abovementioned locality since 1997, it is possible to construct a section of the fluctuation curve, and thus no general conclusions on the development of abundance can be drawn. However, it is possible to state with some tolerance that we did not observe any marked decrease or increase in the population density of *Cameraria ohridella*. We will probably obtain the same result if we carry out an ocular estimate of damage to horse chestnut in the course of more years. Although on the basis of existing studies no detectable effects of *Cameraria ohridella* feeding on the health of horse chestnut were proved (Mrkva 1999), it is not possible to rely on this certainly favourable information in future. Particularly under conditions when attacked trees are further stressed (in towns e.g. by drought, automobile pollutions, etc.) we can expect the dieback of trees. Under situations when traditional antagonists of mining species from the order of *Hymenoptera* have not fulfilled our expectations and populations of *Cameraria ohridella* are not markedly decreased, it is necessary to look for another solution of the present condition. In addition to verified, tested and reliable preparations from the group of chitin creation inhibitors the use of entomopathogenic fungi appears to be promising.

References

GAMS V., 1971. Cephalosporium-artige schimmelpilze (Hyphomycetes). Stuttgart, Gustav Fischer Verlag.

HALL R.A., 1981. The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. In: BURGES H.D. (ed.), Microbial Control of Pests and Plant Diseases 1970–1980. London, Academic Press: 482–498.

KHALIL S.K., BARTOŠ J., TÁBORSKÝ V., 1983. Effect of temperature, pH of the medium and sugar on the germination of spores, development of mycelium and sporulation of the entomopathogenic fungus, *Verticillium lecanii* (Zimm.) Viégas. Agriculture Tropicales et Subtropicales, Universitas Agriculture Praga, *16*: 255–274.

MRÁZ M., 1999. Informace o novém škůdci a způsobu ochrany jírovců a dalších dřevin. Brno, Nadační fond – Národní centrum pro trvale udržitelný rozvoj: 8.

MRKVA R., 1999. Přízrak klíněnky jírovcové obchází Evropou. In: KOLEKTIV, Klíněnka jírovcová, *Cameraria ohridella*. Zvláštní příloha časopisu Veronica, *13*, č. 2. Brno, ČSOP: 12.

Received for publication August 23, 2005

Accepted after corrections November 22, 2005

Infekce přezimujících kukel klíněnky jírovcové *Cameraria ohridella* Deschka et Dimić. houbou *Verticillium lecanii* (Zimmerman)Viégas

T. Samek[1], D. Novotný[2], L. Jankovský[3]

[1] *Ústřední kontrolní a zkušební ústav zemědělský, Brno, Česká republika*

[2] *Výzkumný ústav rostlinné výroby, Praha-Ruzyně, Česká republika*

[3] *Lesnická a dřevařská fakulta, Mendelova zemědělská a lesnická univerzita v Brně, Brno, Česká republika*

ABSTRAKT: V letech 1997–2000 byla sledována populační dynamika klíněnky jírovcové *Cameraria ohridella* Deschka et Dimić na lokalitě v Brně-Modřicích. Dále byla sledována mortalita přezimujících kukel a míra jejich napadení houbami. Mortalita kukel klíněnky jírovcové dosahuje na konci období přezimování téměř 40 %. Na odumřelých kuklách byly zjištěny následující druhy hub: *Verticillium lecanii, Cladosporium herbarum, Cladosporium cladosporioides, Alternaria alternata, Acremonium strictum, Rhizopus stolonifer, Arthrinium phaeospermum, Acremonium* sp. 1. Jedinou zjištěnou houbou s entomofágním statusem byla *Verticillium lecanii.* Tato houba je však známá jako parazit mšic, červců, molic apod.

Klíčová slova: *Cameraria ohridella*; klíněnka jírovcová; jírovec; entomopatogenní houby; *Verticillium lecanii*; mortalita; populační dynamika

Entomopatogenní houby jsou významným mortalitním faktorem, podílejícím se na udržování rovnováhy populací hmyzu. Řada entomopatogenních hub se již využívá jako biologické agens v boji s hmyzími škůdci v zemědělství i lesnictví. Nejznámější jsou zástupci rodu *Beauveria*, především pak kmeny druhu *Beauveria bassiana*, napadající mimo jiné i brouky a motýly. V biologickém boji proti některým lesním škůdcům je aplikována suspenze spor pod obchodním názvem Boverol, resp. Boverosil. Spektrum entomopatogenních hub je však podstatně širší stejně jako spektrum nabízených bioracionálních přípravků proti hmyzu. Vliv entomopatogenních hub na přezimující kukly klíněnky jírovcové *Cameraria ohridella* Deschka et Dimić lze považovat za perspektivní možnost, jak snížit současnou vysokou populační hustotu tohoto škůdce.

Výchozím materiálem pro laboratorní šetření byl opad listí, sbíraný v předjaří. Z opadu byly vypreparovány zimující kukly, které byly předmětem dalšího šetření.

Mortalita kukel klíněnky jírovcové dosahuje na konci období přezimování téměř 40 % (tab. 1 a 2). Rozdíly v míře napadení mezi vzorky z eklektoru a volné plochy, zjištěné na jaře roku 1998, jsou zřejmě důsledkem sporadického a nerovnoměrného výskytu chalcidek (tab. 1). Tito parazitoidé jsou ovšem také napadáni houbami. Zjištěná mortalita na jaře v letech 1998 a 1999 byla srovnatelná (tab. 1 a 2). Mortalita kukel je však významně ovlivněna průběhem zimního období.

V roce 1999 bylo na vzorku dvaceti kukel zjištěno následující spektrum hub: *Verticillium lecanii, Cladosporium herbarum, Cladosporium cladosporioides, Alternaria alternata, Acremonium strictum, Rhizopus stolonifer*. Jediným z izolovaných druhů, který je uváděn jako entomofágní houba, je *V. lecanii*.

Nejčastěji izolovaným druhem bylo *Verticillium lecanii*. Podle Gamse (1971) se jedná o agregát drobnějších druhů.

V roce 2000 bylo na kuklách izolováno 6 druhů hub. Z povrchu kukel byla determinována *Alternaria alternata, Verticillium* sp. 1, *Verticillium* sp. 2, hnědé sterilní mycelium; z povrchově sterilizovaných kukel pak *Alternaria alternata, Arthrinium phaeosphaermum, Acremonium* sp. 1, hnědé sterilní mycelium. Zjištěné druhy jsou spíše saprofytickými druhy, žádný z nich není typickým entomofágem.

Pozorované spektrum hub na přezimujících kuklách z obou let je výrazně odlišné. Jediným druhem, zjištěným v obou letech, je obecně rozšířená *Alternaria alternata*. V obou letech byla rovněž zjištěna přítomnost rodu *Verticillium*. *Verticillium lecanii*, velmi hojné v roce 1999, nebylo v roce 2000 zjištěno.

Verticillium lecanii je relativně dobře prozkoumanou entomopatogenní houbou na stejnokřídlých (*Homoptera*), na většině mšic, červců a molic především v tropických a subtropických oblastech. *V. lecanii* příležitostně hyperparazituje na fytopatogenních houbách, většinou rzích a padlí (Hall 1981). Z kukel motýlů však není uváděno. Virulence *V. lecanii* je spojována s produkcí extracelulárních chitináz. Z toxinů byla detekována produkce cyclodepsipeptidu. Kultivačními podmínkami pro *V. lecanii* se u nás zabývali Khalil et al. (1983). Proti mšicím se většinou používá prostředek s koncentrací $5 \times 10^7 – 1 \times 10^9$ spor na ml s přídavkem smáčedla. Na Slovensku je vyráběn mikrobiální insekticid proti savému hmyzu Vertican. *Verticillium lecanii* se v tropických oblastech, jmenovitě na Kubě a na Floridě, využívá proti štítence *Coccus viridis*. Ve skleníkových kulturách se *V. lecanii* využívá proti *Bemisia tabaci*. Na bázi *V. lecanii* se také vyrábějí některé další přípravky určené proti mšicím (Vertalec), červcům a molicím (Mycotal, Savona). Z hlediska možné parazitace na kuklách klíněnky jírovcové však bude třeba provést řadu dalších testů a pozorování.

Corresponding author:

Doc. Dr. Ing. Libor Jankovský, Mendelova zemědělská a lesnická univerzita v Brně, Lesnická a dřevařská fakulta, Lesnická 37, 613 00 Brno, Česká republika
tel.: + 420 545 134 116, fax: + 420 545 211 422, e-mail: jankov@mendelu.cz