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# Dominating Species of Entomophilous Ascomycetes Anamorphs in West Siberia, Primorsky Krai, and Kyrgyzstan

**G. P. Polovinko[a], O. N. Yaroslavtseva[a], Z. A. Teshebaeva[b], and V. Yu. Kryukov[a]**

[a]*Institute of Systematics and Ecology of Animals, Siberian Branch of the Russian Academy of Sciences, ul. Frunze 11, Novosibirsk, 630091 Russia*
*E-mail: krukoff@mail.ru*
[b]*Adyshev Osh Technological University, ul. Isanova 81, Osh, 714000 Kyrgyzstan*

**Abstract**—The paper studies mycobiota of the dead insects in West Siberia, Primorsky krai, and Kyrgyzstan. Ascomycetes anamorphs of 13 genera are revealed. In all regions *Beauveria bassiana* (Bals.-Criv.) Vuill. dominated comprising on average 68% of the total number of isolates. The fungus hosts list the insects of 7 orders and 32 families with Coleoptera, Lepidoptera, and Hemiptera dominating. The rarely found entomopathogens include *Tolypocladium inflatum* Gams (primarily on Lepidoptera), *Metarhizium anisopliae* (Metschn.) Sorokin (on Coleoptera). The mortality rate of the insects due to micromycetes is observed mainly on enzootic level. The study of the pathogenic properties of the dominating species (*B. bassiana*) show the absence of specificity of its environmental isolates for a number of representatives of Orthoptera, Lepidoptera, Coleoptera, and Diptera.

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Among the microorganisms that are promising as a basis for biopreparations for pest control a special place belongs to anamorphous ascomycetes that earlier were placed in Deuteromycota phylum. Today the representatives of the genera *Beauveria*, *Metarhizium*, *Isaria*, and *Lecanicillium* are used as mycoinsecticides [1]. The experts in Russia and abroad express more and more interest to the entomopathogenic ascomycetes. The subjects of the studies include the role of fungal entomopathogens in the regulation of insect populations, determination of the host range, estimate of the resistance of the infection material to the environment, development of the methods of introduction of mycoinsecticide preparations into biocenoses, synergists of entomopathogenic fungi, and some other problems [2–12].

The efficiency of the fungal preparations is largely conditioned by the initial virulence of isolates as their basis [13–16]. Therefore, the isolates of micromycetes that are isolated from the natural material are always of great interest for the scientists. In addition to that it is suggested that the use of strains and species of entomopathogenic fungi dominating on certain territory can significantly increase the effectiveness of the use of biopreparations in the given area and promote the persistence of the fungi in biocenoses [6, 16–18]. The present study is dedicated to mycobiota of the dead insects collected in the forest cenoses in West Siberia, Primorsky krai, and Kyrgyzstan. The purpose of the work is to reveal the dominating species of ascomycetes anamorphs in the mentioned areas and estimate the pathogenic properties of the dominating species *Beauveria bassiana* (Bals.-Criv.) Vuill.

## MATERIALS AND METHODS

The study was conducted in 2000–2009 in West Siberia (Novosibirsk oblast, Altai krai) and in 2006–2009 in Kyrgyzstan (Urumbash forestry, Karashak and Jergetal forestry, vicinity of Mt. Kordai). We also analyzed the material from Primorsky krai (Lazovskii Nature Reserve, Kedrovaya Pad’ Nature Reserve) collected in 2000–2007 and given at our disposal by Yu. A. Mel’nikova (Institute of Biology and Soil Sciences FEB RAS) and A. A. Makarikov (Institute of Systematics and Ecology of Animals SB RAS). Entomopathogenic fungi were searched for from July to September primarily in forest biocenoses. The dead insects were collected in the forest litter, in the upper soil layer (up to 5 cm) and dead fallen wood. The fungi were isolated into culture according to generally accepted methods [19] with the use of agarized Czapek’s, Waksman’s, and Sabouraud’s nutrient media [20]. For species identification we used the manuals by A. A. Evlakhova [21], E. Z. Koval’ [22], G. R. Lednev et al. [23].

In the laboratory conditions we studied the entomopathogenic properties of 11 isolates of *B. bassiana* dominating in mycobiota of the dead insects. In particular, we studied the properties of West Siberian isolates SSh-98, SSh-99 and SSh-01 isolated from the dead larvae of *Dendrolimus superans sibiricus* Tsch.; KZh-4, K-211 and K3-BP isolated from the dead larvae and imago of *Leptinotarsa decemlineata* Say; NK-2, NK-3 and NK-10 isolated from the mummified caterpillars of *Loxostege sticticalis* L.; SAR-3 and SAR-31 isolated from the dead egg pods of *Calliptamus italicus* L. The cultures of the fungus were grown in Petri dishes on agarized Czapek's and Waksman's media at the temperature of 24°C. The 30-days-old cultures of the fungus were used for infecting the insects.

In the infecting experiments we used the larvae of 3rd and 4th instars of various insect species including *Leptinotarsa decemlineata* Say, *Pieris rapae* L., *Aporia crataegi* L., *Yponomeuta evonymellus* L., *Lymantria dispar* L., *Dendrolimus superans sibiricus* Tsch., *Calliptamus italicus* L., larvae of *Culex* sp., as well as caterpillars of laboratory populations of *Galleria mellonella* L., *Gryllus bimaculatus* Deg., and *Nauphoeta cinerea* Oliv. The majority of the listed test-insects fed on the leaves of their forage plants. The caterpillars of *Galleria mellonella* were given artificial food prepared according to N. A. Tamarina [24], the larvae of *Nauphoeta cinerea* were given cat food, and the larvae of *Gryllus bimaculatus*, a mixture of legumes.

The caterpillars of test-insects were infected using the contact method by placing them in Petri dishes directly on the surface of conidial layer of the fungus isolate for 60 seconds. Each variant of the experiment was repeated 3–5 times using 10–15 individuals each time. The development of mycosis in the test-insects was observed at the temperatures of 20–25°C. The virulence of the isolates were estimated by two parameters: death rate of the infected insects (%) and time of their death (days).

## RESULTS AND DISCUSSION

In the studied regions the mycobiota of the collected cadavers of insects lists ascomycetes anamorphs of 13 genera (Table 1). Such species as *Beauveria bassiana*, *B. brongniartii* (Sacc.) Petch, *Tolypocladium inflatum* Gams, *Isaria farinosa* (Holmsk.) Fr., *I. fumosorosea* Wize, *Metarhizium anisopliae* (Metschn.) Sorokin, and *Lecanicillium* sp. are entomopathogenic fungi, whereas the representatives of the genera *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Helminthosporium*, *Macrosporium*, *Trichoderma*, and *Scopulariopsis* can be conditionally attributed to saprotrophic fungi that more often develop on the insects who died due to other reasons. Although in some cases the species and strains *Aspergillus* and *Fusarium* are experimentally shown to be highly pathogenic for insect [25, 26], it is known that the given fungi usually develops on the insect that died due to mechanic damage to cuticle, bacterioses, viroses, or other entomopathogenic fungi [27, 28].

In all three studied regions *B. bassiana* is a dominating species. Its share of the total number of isolates is on average 68%. The species who host this fungus most often include Lepidoptera with the Macroheterocera group dominating, Pentatomidae of Hemipterans, and Coleopterans with Curculionidae dominating in West Siberia and Chrysomelidae, in Kyrgyzstan (Table 2). During observation period the mortality rate of the insects died of *B. bassiana* was registered mostly on enzootic level, although in some cases the local nidi of mass death of the hosts were noted. In September, 2006, a high death level of imago of *Brachysomus echinatus* (Bonsd.) was registered in the flood-lands of the Bol'shaya Pustynka River (Toguchinskii raion, Novosibirsk oblast). The number of died insects was 1–2 individuals per $m^2$ of forest litter, and no live beetles were found. In August, 2007, a mass death of Pentatomidae was noted in the vicinity of Zherebtsovo Village (Novosibirskii raion) with the number of affected insects being 0.5–1 individuals per $m^2$ of forest litter. In August 2005, accumulations of imago of *Agelastica alni* Baly affected by *B. bassiana* were found in Kyrgyzstan in the flood-lands of the Ak-Buura River (more than 300 dead beetles on the poplar butts). According to B. A. Borisov et al. [29], in 2003 in Primorsky krai a mass death of Acrididae due to this fungus species was noted. Four land Gastropoda overgrown by *B. bassiana* were found in Novosibirsk oblast. Earlier the slugs of *Deroceras caucasicum* Simroth species were found dead of this fungus in Southeast Kazakhstan [5].

The *I. farinosa* entomopathogenic fungus, second in number, is found primarily on the Lepidoptera of the Macroheterocera group, less often on beetles (Coccinellidae, Staphylinidae, Curculionidae) and other insects. This fungus is also likely to have a significant epizootic potential. It is revealed that in some places in Novosibirsk oblast, in particular, near the town of Bolotnoe, the mentioned fungus is present in soil in great amounts. When inoculating caterpillars and pupas of *G. mellonella* with the soil from these places, the mortality rate due to *I. farinosa* and *Isaria* sp. was noted at 75%. The isolated strains of the fungus feature high virulence with 100% death rate of the caterpillars of *G. mellonella* 4–5 days after contact infection.

The rare findings of the pathogens include the *T. inflatum* fungus closely related with *B. bassiana* species. In 1995 this micromycete was found as part of mycobiota of the dead caterpillars of *Loxostege sticticalis* in Novosibirsk oblast [30–32], and in 2007 it was isolated in Kyrgyzstan from the mummified imago of *Agelastica alni*. Of great interest is the finding of *M. anisopliae* in the south of Novosibirsk oblast in 2009 on the larva of Scarabaeidae. Earlier this fungus was reported to be found in West Siberia only in two works [27, 33], with the caterpillars of *Dendrolimus superans sibiricus* and nymphs and imago of *Calliptamus italicus* as hosts. In 2008–2009 *M. anisopliae* caused epizooty in the laboratory population of *Pachnoda marginata* Drury in the Institute of Systematics and Ecology of Animals SB RAS. The fungus was likely to be brought into the laboratory with the soil in which chafers were kept. However, the similar epizooties were recorded in the insectaries in other Russian cities, particularly, in Moscow Zoo [34].

**Table 1.** Ascomycetes anamorphs isolated in West Siberia, Primorsky krai, and Kyrgyzstan

| Insect orders | Number of studied individuals | Isolates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Beauveria bassiana* | *Tolypocladium inflatum* | *Isaria farinosa* | *Metarhizium anisopliae* | *Lecanicillium* sp. | *Alternaria* sp. | *Aspergillus* sp. | *Penicillium* sp. | Other micromycetes |
| | | | | | Kyrgyzstan | | | | | |
| Lepidoptera | 28 | 6 | – | 1 | – | – | 6 | 4 | 4 | 7[1] |
| Coleoptera | 16 | 12 | 1 | – | – | – | 1 | – | 1 | 1[2] |
| Diptera | 2 | – | – | – | – | – | – | – | 1 | 1[3] |
| Share, % | 100 | 39 | 2 | 2 | 0 | 0 | 15 | 9 | 13 | 20 |
| | | | | | West Siberia | | | | | |
| Orthoptera | 5 | 4 | – | – | 1* | – | – | – | – | – |
| Hemiptera | 17 | 15 | – | – | – | – | – | 1 | 1 | – |
| Homoptera | 1 | 1 | – | – | – | – | – | – | – | – |
| Lepidoptera | 56 | 35 | 5 | 10 | – | 2 | – | 3 | 1 | – |
| Coleoptera | 44 | 38 | – | 3 | 1 | – | – | 1 | 1 | 3[4] |
| Hymenoptera | 10 | 9 | – | – | – | – | – | – | 1 | – |
| Share, % | 100 | 75 | 4 | 10 | 1 | 1 | 0 | 4 | 3 | 2 |
| | | | | | Primorsky krai | | | | | |
| Orthoptera | 4 | 4 | – | – | – | – | – | – | – | – |
| Hemiptera | 3 | 3 | – | – | – | – | – | – | – | – |
| Coleoptera | 3 | 3 | – | – | – | – | – | – | – | – |
| Lepidoptera | 2 | 2 | – | – | – | – | – | – | – | – |
| Hymenoptera | 8 | 6 | – | – | – | 1 | – | – | – | 1[5] |
| Share, % | 100 | 90 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 |

[1] *Fusarium* sp., *Macrosporium* sp., *Helminthosporium* sp. on caterpillars of *Lymantria dispar*. [2] *Isaria fumoso-rosea* on Coccinellidae. [3] *Scopulariopsis* sp. on Brachycera, Cyclorrhapha. [4] *Beauveria brongniartii* on *Pythodepressus* L. (isolated by B. A. Borisov, Severtsov Institute of Ecology and Evolution RAS), *Trichoderma* sp. on Carabidae, *Scopulariopsis* sp. on larva of *Leptinotarsa decemlineata*. [5] *Scopulariopsis* sp. on *Formica* sp.

* Isolate Mak-1 is isolated by G. R. Lednev and M. V. Levchenko ( All-Russia Institute of Plant Protection, Russian Academy of Agricultural Sciences) [33].

**Table 2.** Host-insects of *Beauveria bassiana* noted in West Siberia, Primorsky krai and Kyrgyzstan in 2000–2009

| Taxon | Abundance | Region | Taxon | Abundance | Region |
|---|---|---|---|---|---|
| Hemiptera | | | Lepidoptera | | |
| Miridae | + | S | Cossidae: *Cossus* sp. | + | S |
| Pentatomidae | +++ | S P | Pyralidae: | | |
| Orthoptera | | | *Loxostege sticticalis* L. | ++ | S |
| Acrididae: | | | Pieridae: *Aporia crataegi* L. | + | S |
| *Calliptamus italicus* | + | S | Sphingidae: *Mimas tiliae* L. | + | S |
| *?* sp. | + | P | Geometridae | ++ | S |
| Homoptera | | | Thyatiridae | + | S |
| Cicadidae | + | S | Lasiocampidae: | | |
| Coleoptera | | | *Euthrix potatoria* L. | + | P |
| Carabidae | + | S K | *Dendrolimus superans* Butl. | + | S |
| Staphylinidae | + | S K | Notodontidae: *Notodonta* sp. | + | S |
| Elateridae | + | S K | Lymantriidae: | | |
| Silphidae: *Phosphuga atrata* L. | + | K | *Lymantria dispar* L. | ++ | P K |
| Coccinellidae | + | S K | Noctuidae | + | S |
| Cisidae | + | S | Hymenoptera | | |
| Tenebrionidae | + | K | Cimbicidae: | | |
| Mordellidae | + | K | *Cimbex femorata* L. | + | S |
| Cerambycidae | + | K | *?* sp. | + | S |
| Scarabaeidae: *Melolontha* sp. | + | S | Vespidae | + | S P |
| Chrysomelidae: | | | Icneumonoidae | + | S |
| *Leptinotarsa decemlineata* Say | + | S | Formcidae: | | |
| *Agelastica alni* Baly | +++ | K | *Mirmica* sp. | + | S |
| *?* sp. | + | S | *Formica* sp. | + | S P |
| Curculionidae: | | | Diptera | | |
| *Brachysomus echinatus* Bonsd. | +++ | S | Asellidae | + | S |
| *Polydrusus undatus* F. | + | S | Muscidae | + | S |
| *Eudipnus mollis* Strom | + | S | | | |
| *Phyllobius pomaceus* Gyll. | + | K | | | |
| *?* sp. | ++ | S P K | | | |

Note: +, less 5%; ++, 5 to 10%; +++, more than 10%. S, West Siberia; P, Primorsky krai; K, Kyrgyzstan.

To study the virulence we selected 11 West Siberian isolates of the dominating fungus *B. bassiana*. These isolates differed in the source of isolation, i.e., the primary host (Table 3). The laboratory experiments on infecting the test-insects showed that all studied isolates were highly virulent for the majority of the species of test-insects: caterpillars of *Galleria mellonella*, *Yponomeuta evonymellus*, *Aporia crataegi*, *Leptinotarsa decemlineata*, and *Calliptamus italicus*. The caterpillars of the listed species died in 3–8 days. The larvae of *Gryllus bimaculatus*, *Dendrolimus superans sibiricus*, *Lymantria dispar*, and other species are less sensitive to the isolates of *B. bassiana*, which is confirmed by later time of their death. The caterpillars of *Lymantria dispar* died in 13–18 days, larvae of *Culex* sp., in 12–22 days, caterpillars of *Dendrolimus superans sibiricus*, in 18–22 days, and the larvae of *Gryllus bimaculatus*, in 25–30 days. We should note that the isolates from a certain host are not necessarily more virulent for this species of insect. Thus, the isolates from the cadavers of *Dendrolimus superans sibiricus* were less virulent for the caterpillars of the host-insect, but more virulent for the other species of insects. The most virulent for *Lymantria dispar* were the strains isolated from *Calliptamus italicus*, not from Lepidoptera. The similar tendency, i.e., absence of specificity of strains of *B. bassiana* for different hosts, was earlier illustrated by *Leptinotarsa decemlineata*, *Calliptamus barbarus*

**Table 3.** Virulent properties of West Siberian isolates of the fungus *Beauveria bassiana* isolated from dead insects of different systematic groups

| Species of test-insect | Host-insect, isolates | | | |
|---|---|---|---|---|
| | *Calliptamus italicus* SAR-3, SAR-31 | *Leptinotarsa decemlineata* 211, KZh-4, K3-BP | *Loxostege sticticalis* NK-2, NK-3, NK-10 | *Dendrolimus superans* SSh-98, SSh-99, SSh-01 |
| *Nauphoeta cinerea* | 18 – 20/50 | – | 0 | – |
| *Gryllus bimaculatus* | – | 25 – 30/80 – 100 | 0 | – |
| *Calliptamus italicus* | 7 – 8/100 | – | – | 10/30 – 40 |
| *Leptinotarsa decemlineata* | — | 3 – 7/100 | 5 – 9/100 | — |
| *Yponomeuta evonymellus* | 3 – 4/100 | 4 – 7/100 | 2 – 4/100 | 4 – 7/100 |
| *Galleria mellonella* | | 5 – 9/100 | 7 – 9/100 | – |
| *Aporia crataegi* | – | 3 – 5/100 | 5 – 7/100 | 4 – 5/100 |
| *Pieris rapae* | 10/100 | – | 8 – 10/100 | – |
| *Dendrolimus superans* | — | 20 – 22/100 | 7 – 12/80 – 100 | 12 – 22/80 – 100 |
| *Lymantria dispar* | 13/100 | – | 0 | 18/35 |
| *Culex* sp. | | 13 – 14/50 | 10 – 13/100 | 12 – 22/100 |

Note: Numerator, time of death of test-insect caterpillars, days; denominator, share of death of test-insect caterpillars, %.

Costa and *Locusta migratoria* L. in the series of laboratory experiments [8]. It was also established that the isolates from the same geographical points and the same insect species can differ greatly in their virulence. For instance, the isolates of *T. inflatum* from the dead caterpillars of *Loxostege sticticalis* were more virulent for other insects (*Galleria mellonella*, *Yponomeuta evonymellus*, *Pieris rapae*, *P. brassicae*, *Leptinotarsa decemlineata*) than for the caterpillars of the host-insect [32]. The similar tendency is noted for *B. bassiana* by T. K. Kal'vish [27], A. S. Kamenova [35], and other researchers.

Therefore, the composition of micromycetes in mycobiota of the dead insects collected in the studied regions differs little from that in other regions of Russia and CIS. The entomopathogenic species of fungi of the genera *Beauveria*, *Isaria*, *Lecanicillium*, *Scopulariopsis*, *Fusarium*, *Penicillium*, *Aspergillus* that we determined are found within the mycobiota of the dead insects collected in the forest cenoses in Tomsk, Kemerovo, Irkustk oblasts, Altai Republic, and Trans-Ili Alatau [15, 16, 25, 35, 36]. At the same time we found the species of entomopathogenic fungi rare for these territories, *M. anisopliae*, *T. inflatum*. The mycobiota of the insects in the studied areas was dominated by *B. bassiana*. Its hosts included the insects of 32 families and 7 orders, as well as Gastropoda. The death of the insects due to this pathogen was observed primarily on enzootic level. The isolates of *B. bassiana* did not show any selectiveness for certain insects. They are characterized by high virulence for not only the primary host-insects, but also some other insect species. The isolates can be used for preparations with mycoinsecticidal properties for the insects of various orders.

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