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Host range and recorded distribution of the fungal pathogen *Entomophaga grylli* (Entomophthoromycota: Entomophthorales) in Kazakhstan

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Abstract

In this paper for the first time we summarized both the existing literature data and our own findings on the occurrence of the infections caused by the fungus *Entomophaga grylli* (Fresenius) A. Batko, 1964 (Entomophthoromycota: Entomophtorales) among short-horned Orthoptera (Caelifera) in Kazakhstan. Almost 800 insect cadavers exhibiting signs of infection by *E. grylli* were collected in 7 out of 14 provinces (oblasts) of Kazakhstan in 2000-2016, with confirmed identification of the fungal pathogen. They belonged to 18 genera and 28 species from two families, Pamphagidae and Acrididae, and from 6 subfamilies within the latter family. A discussion summarizing the current knowledge of the host range of the fungus in Kazakhstan and its potential for the use in biological control programs is provided.

Key words

entomopathogen, grasshoppers, locusts, biological control

Introduction

Locusts and grasshoppers are among the most economically important agricultural pests in Kazakhstan. During outbreaks, they can inflict severe damage to crops and rangeland. For example, in 1999, infestations of the Italian locust Calliptamus italicus (Linnaeus) (Orthoptera: Acrididae) in Northern and Central provinces destroyed 220,000 ha of grain crops with an estimated value of USD 15 million (Khasenov 2001). To prevent agricultural losses, every year immense areas of locust and grasshopper infestations in Kazakhstan are treated with broad-spectrum pesticides. In 2000, the area of locust and grasshopper control in the country exceeded 8.1 million ha (Khasenov 2001), which is a world record of anti-acridid treatments per country per year. Such large-scale applications of insecticides have not only very high economic but also tremendous environmental costs (Latchininsky et al. 2002). Growing concerns about environmental impacts of locust and grasshopper treatment programs with chemical insecticides in Kazakhstan evoked interest in alternative control strategies involving natural regulators of pest populations (Temreshev & Khasenov 2004). Fungal pathogens are among the most promising natural control agents for locusts and grasshoppers worldwide (Lomer & Prior 1991, Goettel 1992) and in Kazakhstan in particular (Temreshev 2003, Temreshev & Childebaev 2011, Temreshev et al. 2012, 2015, 2016).

Despite the fact that there are over 700 known species of entomopathogenic fungi worldwide (Goettel 1992), only very few of them are proven to infect grasshoppers and locusts (Orthoptera: Acridoidea) (Prior & Greathead 1989). Among them, the cosmopolitan fungus *Entomophaga grylli* (Fresenius) A. Batko, 1964 is famous for causing spectacular natural epizootics on different continents: in Africa

(Skaife 1925, Lepesme 1938, Chapman & Page 1979), Australia (Milner 1978), North America (Treherne & Buckell 1924, Walton & Fenton 1939, Pickford & Riegert 1964, MacLeod & Müller-Kögler 1973, McDaniel & Bohls 1984, Erlandson *et al.* 1988, Valovage *et al.* 1984, Valovage & Nelson 1990), Central America (Sánchez-Pena 2000) and South America (Marchionatto 1934, Fresa 1971, Méndez-Sanchéz *et al.* 2009, Pelizza *et al.* 2010). The insects infected by *E. grylli* exhibit distinctive and unique signs at the advanced stages of the disease. They climb on top of plants where they die with head pointing upwards and legs tightly clinging around the stems (Fig. 1). Because of such characteristic posturing, the disease caused by *E. grylli* received the term "summit disease."

In Eurasia, the infections caused by E. grylli attracted special attention of the Father of Modern Acridology, Sir Boris Uvarov, who published his detailed observations on fungal epidemics of grasshoppers and locusts in southern Russia a century ago (Uvarov 1913). However, cases of acridid fungal diseases attributable to E. grylli were reported from Russia even earlier (Koeppen 1870, Porchinsky 1894) and probably as early as in the 18th century (Pallas 1771-1801). The fungus is known to attack several economic species of locusts and grasshoppers on a vast geographic scale of the former Soviet Union: in northern Caucasus (Zhdanov 1934), Volga region (Batko 1957), Siberia (Vinokurov 1949, Karelina 1961, Latchininsky 1995, Ogarkov & Ogarkova 2000), Turkmenistan (Tokgaev 1973), Uzbekistan (Gapparov 1988), and Georgia (Abashidze et al. 1998). Besides the ex-USSR, other reports of E. grylli in Eurasia come from Thailand (Roffey 1968), China (Chen & Liu 1995, Jia 2011), Vietnam (Weiser et al. 1985), India (Gupta et al. 2011), Palestine (Ali-Shtayeh et al. 2003) and Iran (Ghazavi et al. 2003). Regarding Kazakhstan, the records of E. grylli infections were fragmentary (Vasil'ev 1962; Evlakhova & Shvetsova 1965, Evlakhova 1974, Nasyrova 1992, 1995) until recently, when regulatory potential of fungal diseases of locusts and grasshoppers was re-examined (Temreshev 2003). Yet the relevant information appeared only partially in conference proceedings or unpublished reports which are not available in English (Temreshev & Khasenov 2004, Temreshev & Childebaev 2011, 2012, Temreshev et al. 2012). In this paper for the first time we summarized both the existing literature data and our own findings on the occurrence of the infections caused by E. grylli among short-horned Orthoptera (Caelifera) in Kazakhstan. A discussion summarizing the current knowledge of the host range of the fungus in Kazakhstan and its potential for the use in biological control programs is provided.

Materials and methods

Data were collected in 7 out of 14 provinces (oblasts) of Kazakhstan in 2000-2012. No systematic effort was made to sample

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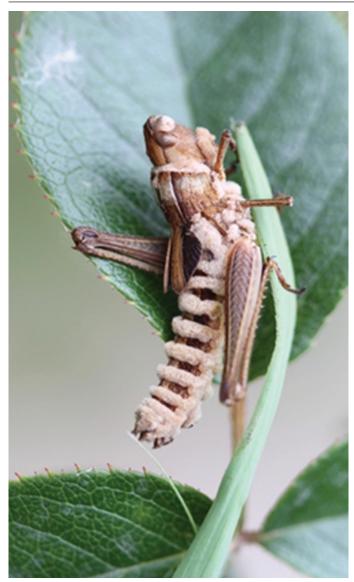


Fig 1. Chorthippus angulatus killed by Entomophaga grylli in Kazakhstan. Photo V.L. Kazenas. Reproduced by permission.

all of Kazakhstan. In most cases the dead insect collections were made in the areas of high-density grasshopper and locust populations and known disease occurrence. Dead insects exhibiting the "summit disease" signs were collected mostly from the stems of various plants, placed into plastic or glass vials sterilized with 70% EtOH, and stored at +4°C for subsequent examination. In case there was no visible mycelium on the insect body, the specimens were placed into a "wet chamber" until the sporulation started. The "wet chamber" represented a Petri dish bottom with a round piece of filter paper soaked with distilled water and closed with a Petri dish lid (Evlakhova & Shvetsova 1965, Issi *et al.* 1993). Identification of the pathogen was done by examining the conidia under the microscope MBI-15 (magnification 40× and 90×), measuring the spore size by an ocular micrometer MOB-1-15×, and following the keys of the "Guide to entomophilous fungi of the USSR" (Koval 1974).

To ensure the proper identification of Caelifera host species of the fungus, sweep-net samples of live grasshoppers were collected using the standard entomological net of 30 cm diameter. The insects were then killed in a killing jar using ethyl acetate and subsequently identified in the lab using the keys from "Grasshoppers and Locusts"

of Kazakhstan, Central Asia and Adjacent Territories" by Latchininsky et al. (2002). Acridoidea classification from the above publication was followed for families and subfamilies.

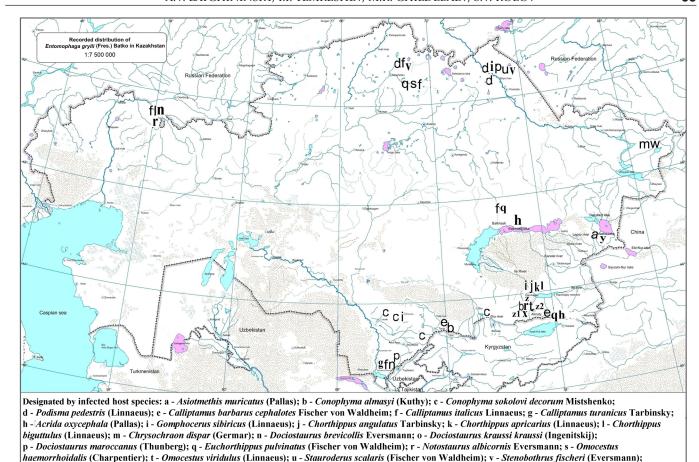
Results and discussion

In total almost 800 cadavers of grasshoppers and locusts (Caelifera) exhibiting signs of infection by *E. grylli* were collected, with confirmed identification of the fungal pathogen. They belonged to 18 genera and 28 species from two families, Pamphagidae and Acrididae, and from 6 subfamilies within the latter family (Table 1). All infected insects were adults or last (5th) instar nymphs except for one 4th instar nymph of *Euchorthippus pulvinatus* (Fischer von Waldheim) (Orthoptera: Acrididae: Gomphocerinae). Data from these collections were used to produce a distribution map of *E. grylli* in Kazakhstan (Fig. 2). In the vast majority of cases the prevalence of the *E. grylli* mycosis in the host population was low except for cases of *Podisma pedestris* (Linnaeus) (Orthoptera: Acrididae: Melanoplinae) and *Gomphocerus sibiricus* (Linnaeus) (Orthoptera: Acrididae: Gomphocerinae) in the Pavlodar province of NE Kazakhstan in June 2004.

The present study is the first attempt to summarize existing literature and original field data on Caelifera host range and geographic distribution of *E. grylli* mycoses in Kazakhstan. The data are by no means exhaustive; undoubtedly, more comprehensive surveys will complement the list of susceptible host species and geographic locations of *E. grylli* occurrences. Yet even this, admittedly fragmentary information provides valuable insights regarding the host range and distribution of this important pathogen in Kazakhstan.

First, it was of interest to find out if E. grylli affects pest and/or rare Caelifera species. The fauna of Caelifera in Kazakhstan includes 264 species and subspecies (Latchininsky et al. 2002) with about 20 of those being recurrent agricultural pests (Mistchenko 1972, Nurmuratov et al. 2000). None of the Kazakhstani Caelifera species are currently designated as threatened or endangered; however, there are 45 species and 16 subspecies endemic to Kazakhstan, some of which are extremely rare (Childebaev 2000). Based on our study, 14 out of 28 species affected by *E. grylli* in the field were agricultural pests (designated by an asterisk in Table 1), including all three locusts species inhabiting Kazakhstan: Locusta migratoria migratoria Linnaeus (Orthoptera: Acrididae: Oedipodinae), Dociostaurus maroccanus (Thunberg) (Orthoptera: Acrididae: Gomphocerinae), and C. italicus. For the latter locust species, E. grylli is well known as one of the most important population regulators (Uvarov 1928, Evlakhova & Shvetsova 1965). It is interesting to note though that according to Uvarov (1977), the Italian locust mycoses caused by E. grylli were never reported from dry steppes of Kazakhstan because of unfavorable arid conditions for the fungus (p. 459). Our findings from several sites in dry steppes of Central Kazakhstan (Table 1) contradict this assertion. Finally, none of the E. grylli hosts from our collections were Kazakhstani endemics, probably because of their lower population densities compared to more common and abundant species.

Another intriguing question is the taxonomic status of *E. grylli* in Kazakhstan. Although the fungus is cosmopolitan, beyond the North American continent its taxonomy is studied insufficiently (Humber 1989). The fungus is known to be represented by several distinctive pathotypes which differ in host ranges, isozyme banding patterns, growth requirements, life cycles, and DNA characteristics (Soper *et al.* 1983, Humber 1989, Bidochka *et al.* 1995). There is a growing molecular biological evidence that these pathotypes are distinctive species (Bidochka *et al.* 1995, Casique-Valdez *et al.* 2012).



w - Stenobothrus eurasius Zuboyski; x - Epacromius tergestinus (Charpentier); v- Locusta migratoria migratoria (Linnaeus); z - Oedaleus decorus Germar;

Fig 2. Map of Entomophaga grylli distribution in Kazakhstan.

zl - Oedipoda caerulescens (Linnaeus); z2 - Oedipoda miniata (Pallas).

suggest that there exists a European pathotype, or E. grylli (sensu stricto) (Carruthers et al. 1997), which may occur in Kazakhstan (R. Humber, pers. comm. 2013). Without further analyses using molecular techniques it is impossible to assert which pathotype(s) occur(s) in Kazakhstan. Since some of the studied pathotypes exhibit differences in host ranges, the question of the pathotype designation has important practical implications for the use of *E*. grylli in biological control of pest acridids. Preliminary evidence from Kazakhstan (unpub. data) suggest that inoculations by E. grylli spores obtained from one grasshopper species were successful in species across several Acrididae subfamilies, which reveals a broad host range of the pathogen.

Our investigation showed that many pest species were susceptible to E. grylli mycoses, which makes the fungal pathogen a potential candidate for developing a biological product for locust and grasshopper control. However, it is well known that E. grylli does not produce reproductive stages on most complex solid or liquid artificial media (Evlakhova & Shvetsova 1965, Ramoska et al. 1988, Bidochka et al. 1995, Pell et al. 2001, Geshtovt 2002), which makes impractical its mass production for biological control purposes (Bidochka & Khachatourians 1991, Carruthers et al. 1997). Despite some progress in growing *E. grylli* hyphae *in vitro* (Sánchez-Peña 2005), the production of conidia has not been successful to and the steppe (Temreshev & Childebaev 2012). This observation date. Under such circumstances, it is conceivable to use E. grylli in suggests that E. grylli in Kazakhstan can infect its acridid hosts across

E. grylli was described by Fresenius from Europe, so some specialists tion of insects with fungal spores in the lab and their subsequent releases in the field, in order to create areas of higher-than-average level of pathogen infection (Carruthers et al. 1997).

As elsewhere in the world, grasshoppers and locusts infected by E. grylli in Kazakhstan were found clinging to tops of plant stems (Fig. 1). Some specialists consider this behavior as an attempt to thermoregulate and increase the body temperature above the thermal limit of E. grylli (Kemp 1986) while others suggest that the fungus causes this behavioral modification to increase the aerial dissemination of spores (Carruthers et al. 1997). Grasshopper and locust cadavers exhibiting the "summit disease" signs were most frequently found in damp areas with dense herbaceous vegetation such as roadside ditches, moist meadows and hayfields, intermittent waterways in pastures and weedy perimeters of cropland. This is consistent with findings in other geographic areas indicating that areas with higher humidity create favorable conditions for E. grylli mycoses; for the same reason, the higher prevalence of E. grylli is usually correlated with wetter than usual years (Evlakhova & Shvetsova 1965, Erlandson et al. 1988, Valovage & Nelson 1990, Packham et al. 1993, Carruthers et al. 1997, Laws et al. 2009, Pelizza et al. 2010). Interestingly, the two epizootics of Podisma pedestris and Gomphocerus sibiricus developed in notably drier conditions, namely at the forest borders, i.e. the interface between the forest augmentative biological control strategy, which consists in inocula- a relatively wide moisture gradient, which is an important trait for a potential biocontrol agent.

To sum up, our investigations showed that: a) *E. grylli* attacks a wide range of Caelifera hosts, including 28 species from 6 subfamilies of the Acrididae family in Kazakhstan; b) the majority of recurrent agricultural and/or rangeland pests, including 3 locusts, are susceptible to *E. grylli*; and c) *E. grylli* mycoses occur not only in humid but also in relatively dry sites. All the above-mentioned traits make the Kazakhstani strain(s) of *E. grylli* a promising candidate for use in grasshopper and locust augmentative biocontrol program through inoculative releases of this native pathogen.

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Table 1. Annotated list of Caelifera hosts of Entomophaga grylli in Kazakhstan. Pest species are marked with an asterisk (*).

#	Caelifera host species	N	Date	Geographic location
	Family Pamphagidae			
	Subfamily Pamphaginae	2	June 06, 2002	CE Varakhatan Almatu prayinga Alakal diatrict
	Asiotmethis muricatus (Pallas, 1771)	3	June 06, 2002	SE Kazakhstan, Almaty province, Alakol district
	Family Acrididae			
	Subfamily Conophyminae			
	Conophyma sokolovi decorum Mistshenko, 1951	10	May 14, 2012	S Kazakhstan, Zhambyl province, Kordai pass
		20	May 15, 2012	S. Kazakhstan, Zhambyl province, near city Taraz S. Kazakhstan, South Kazakhstan province, town im. T. Ryskulova
		30	May 25, 2012	3. Razakiistaii, 30uul Razakiistaii piovince, towii iii. 1. Ryskuiova
		30	141dy 25, 2012	S. Kazakhstan, South Kazakhstan province, near city Kentau
		10	May 17, 2012	S. Kazakhstan, South Kazakhstan province, Karatau Mtns, Tassarai gorge
		15	May 17-18, 2012	
	Conophyma almasyi (Kuthy, 1905)	3	July 8, 2015	SE Kazakhstan, Almaty province, Ile-Alatau months, Big Almaty gorge
	Subfamily Melanoplinae	10	1 1 7 2002	
4*	Podisma pedestris (Linnaeus, 1758)	10 350	July 7, 2002 June 25, 2004	N. Kazakhstan, Akmola province, Zerendin district N. Kazakhstan, Pavlodar province, Kachiry district
		30	June 26, 2004	N. Kazakhstan, Pavlodar province, Raciniy district N. Kazakhstan, Pavlodar province, Irtysh district
	Subfamily Calliptaminae	30	june 20, 200 i	14. Nazakiistari, Faviodar province, iriyin district
*	Calliptamus barbarus cephalotes Fischer von	3	May 27, 2000	S. Kazakhstan, Zhambyl province, Zhualin district
	Waldheim, 1846	6	June 25, 2000	SE Kazakhstan, Almaty province, Kaskelen district
*	Calliptamus italicus (Linnaeus, 1758)	15	July 7, 2002	N. Kazakhstan, Akmola province, Zerendin district
		12	July 12, 2002	N. Kazakhstan, Akmola province, Enbekshilder district
		3	July 13, 2004	Central Kazakhstan, Karaganda province, Bektau-Ata Mtn
		?	1991	W Kazakhstan, West Kazakhstan province, Aksuy district
*	Calliptamus turanicus Tarbinsky, 1930	5	May 09, 2002	S Kazakhstan, South Kazakhstan province, Saryagash district
	Subfamily Acridinae			1 70
	Acrida oxycephala (Pallas, 1771)	1	June 25, 2000	SE Kazakhstan, Almaty province, Kaskelen district
		5	July 08, 2004	Central Kazakhstan, Karaganda province, Aktogai district
	Subfamily Gomphocerinae			
	Chorthippus angulatus Tarbinsky, 1927	9	June 17, 2011	SE Kazakhstan, Almaty province, Kerbulak district, foothills of Katutau
	(Fig. 1)	4	June 24, 2011	Mtns., 135 km from Saryozek town. S Kazakhstan, South Kazakhstan province, Karatau Mtns., River Ikansu
		4	June 24, 2011	valley
0	Chorthippus apricarius (Linnaeus, 1758)	6	June 17, 2011	SE Kazakhstan, Almaty ptovince, Kerbulak district, foothills of Katutau
	,		,	Mtns., 135 km from Saryozek town
1 *	Chorthippus biguttulus (Linnaeus, 1758)	?	1991	W Kazakhstan, West Kazakhstan province, Aksu district
		12	June 17, 2011	SE Kazakhstan, Almaty province, Kerbulak district, foothills of Katutau
				Mtns., 135 km from Saryozek town
2*	Chrysochraon dispar (Germar, 1835)	3	July 7, 2013	E Kazakhstan, East Kazakhstan province, Abay district
3*	Dociostaurus brevicollis Eversmann, 1848	?	1991	W Kazakhstan, West Kazakhstan province, Aksu district
4*	Dociostaurus kraussi kraussi (Ingenitskij, 1897)	8	June 25, 2004	N. Kazakhstan, Pavlodar province, Kachiry district
5*	Dociostaurus maroccanus (Thunberg, 1815)	8	May 05, 2002	S Kazakhstan, South Kazakhstan province, Saryagash district
	,			• • • •
6	Euchorthippus pulvinatus (Fischer von Waldheim,		July 13, 2004	Central Kazakhstan, Karaganda province, Bektau-Ata Mtn,
	1846)	3	June 24, 2001	SE Kazakhstan, Almaty province, Kaskelen district
7*	Gomphocerus sibiricus (Linnaeus, 1767)	150	June 25, 2004	N Kazakhstan, Pavlodar province, Kachiry district
Q	Natactaurus alhicarnis Evaramana 1949	10	June 25, 2012	SE Kazaldatan Almatu province Ili district
8	Notostaurus albicornis Eversmann, 1848	10 ?	June 25, 2012 1991	SE Kazakhstan, Almaty province, Ili district W Kazakhstan, West Kazakhstan province, Aksu district
9	Omocestus haemorrhoidalis (Charpentier, 1825)	10	July 7, 2002	N Kazakhstan, Akmola province, Zerendin district
0	Omocestus viridulus (Linnaeus, 1758)	6	July 8, 2015	SE Kazakhstan, Almaty province, Ile-Alatau months, Kazachka gorge
1*	Stauroderus scalaris (Fischer von Waldheim,	12	June 25, 2004	N Kazakhstan, Pavlodar province, Kachiry district
	1846)	_	,,	, , _k
2*	Stenobothrus fischeri (Eversmann, 1848)	4	July 7, 2002	N Kazakhstan, Akmola province, Zerendin district
		8	June 25, 2004	N Kazakhstan, Pavlodar province, Kachiry district
3	Stenobothrus eurasius Zubovski, 1898	1	July 7 2013	E Kazakhstan, East Kazakhstan province, Abay district
4	Subfamily Oedipodinae	2	I 24 2021	CF Wardhata Alaston V 1 1 1 1 1 1
4	Epacromius tergestinus (Charpentier, 1825)	2	June 24, 2001	SE Kazakhstan, Almaty province, Kaskelen district
5*	Locusta migratoria migratoria Linnaeus, 1758	6	July 06, 2002	SE Kazakhstan, Almaty province, Alakol district
_	IIII IIII IIII IIII IIII IIII II	Ü	,, 00, 2002	
6*	Oedaleus decorus Germar, 1817	5	June 25, 2012	SE Kazakhstan, Almaty province, Ili district
_				
7	Oedipoda caerulescens (Linnaeus, 1758)	3	June 22, 2001	SE Kazakhstan, Almaty province, Kaskelen district
8	Oedipoda miniata (Pallas, 1771)	2	September 12,	SE Kazakhstan, Almaty province, Almaty city
	pour	-	2014	

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