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Dedicated to Professor Maria Ławrynowicz on the occasion of the 45th anniversary of her scientific activity

Melampsoridium hiratsukanum – invasive rust species in Lithuania, and its co-occurrence with eriophylid mite

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An invasive East Asian rust fungus *Melampsoridium hiratsukanum,* obligate biotroph belonging to *Pucciniastraceae*, Pucciniales (Basidiomycota) is found widely spread on leaves of *Alnus incana* in eastern, central and southern parts of Lithuania. On *Alnus glutinosa* this fungus is rare, sometimes occurring with an alder leaf pest, a microscopic eriophyid gall mite *Acalitus brevitarsus.* Information on the distribution patterns, ecological and morphological characters of this neomycete is given.

Key words: Pucciniales, biotroph, *Melampsoridium hiratsukanum*, *Alnus*, *Acalitus brevitarsus*, Lithuania

INTRODUCTION

Majority of pathogenic neomycetes are classified as having considerable negative ecological and economic impact (Pimentel et al. 2001; Desprez-Loustau et al. 2007, 2010; NOBANIS 2007). Alien fungal diseases of woody plants represent a serious risk to forest ecosystems and growing plantations. In the mid-1990ies epidemic of a new foliar rust fungus affecting alder trees appeared in the Baltic region, in Estonia and Finland (Põldmaa 1997; Kurkela et al. 1999) and started to spread rapidly in Europe (Hantula, Scholler 2006). The agent was identified as *Melampsoridium hiratsukanum* S. Ito ex Hirats. based on morphological characterization and later was confirmed by molecular data (Hantula et al. 2009).

In Lithuania, the fungus appeared at the same time, the first specimen was collected in 1997 in Ukmergė district on *Alnus incana* (L.) Moench (herbarium data, BILAS 31446). Originally M. *hiratsukanum* was described in 1927 on *Alnus hirsuta* (Spach.) Rupr. in Japan (Hiratsuka 1927) and was reported as native species in Far East Asia (Kuprevich, Tranzschel 1957; Hiratsuka et al. 1992; Gjaerum 1996; Chen 2002; Cho, Shin 2004; Kobayashi 2007). *M. hiratsukanum* represents a heteroecious

rust fungus belonging to Pucciniastraceae (Pucciniales, Basidiomycota) characterized by macrocyclic host-alternating life mode. Its uredinial and telial stages occur on *Alnus* spp., while the aecial stage develops on *Larix* spp. (Kuprevicz, Tranzschel 1957, Kaneko, Hiratsuka 1981; Roll-Hansen, Roll-Hansen 1981).

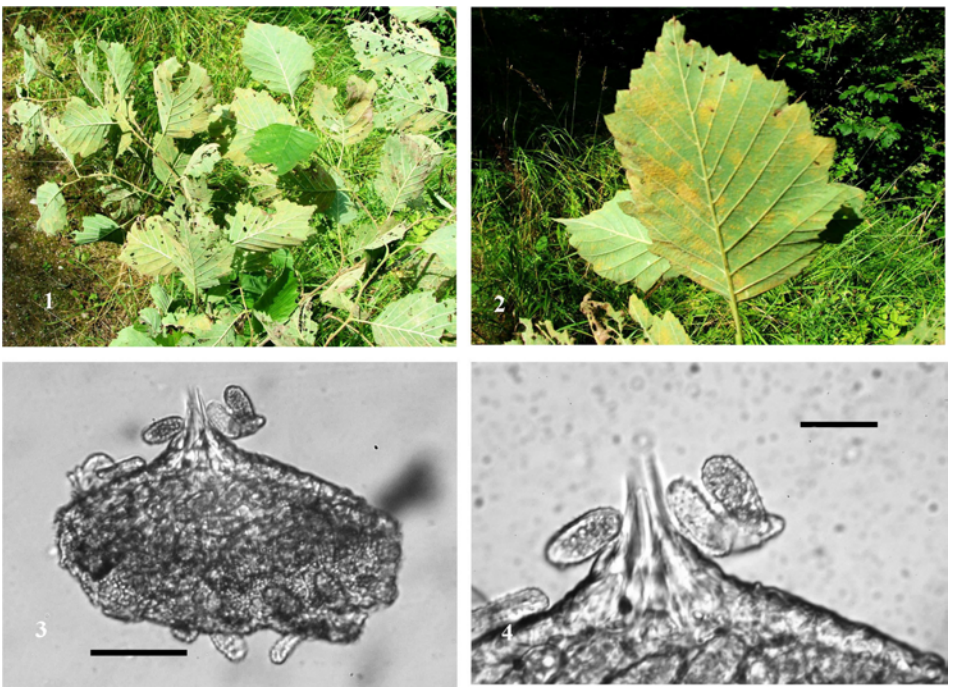
The detailed comparison of urediospore morphology and r-DNR sequence analysis of the ITS region showed that European population of *M. hiratsukanum* is conspecific with population of this fungus from Eastern Asia and that both populations belong to a single palearctic population (Hantula et al. 2009). In Europe this aggressive rust commonly cause considerable damage to foliage of grey alder, but may also infect more resistant black alder and cause serious problems in forest stands mixed with larch (Kurkela et al. 1999; Hantula et al. 2009). Introduction of this pathogen may be a result of an unintentional human activity such as transporting propagative plant material and seeds around the world or because of natural climatic factors. Natural migration of *M. hiratsukanum* could be explained by continuity of its main (*Alnus* spp.) and alternate (*Larix* spp.) host distribution from the Far East, where it inhabits native for Asia Manchurian alder (Hiratsuka et al. 1992), to Siberia (Kuprevicz, Tranzschel 1957), Fennoscandia, Baltic region and further to Europe, where it occurrs on Eurasian grey and native for Europe black alders as well as on various (Dahurian, Siberian, European) larches (Põldmaa 1997; Kurkela et al. 1999; Hantula et al. 2009; Lilja et al. 2011). Changing climate with milder winters and increased precipitation probably also stimulated invasion and rapid spread of this neomycete in the new regions on new hosts during the last decades.

Nowadays, *M. hiratsukanum* is widely spread in many European countries and is placed in the lists of important invasive species (Hantula, Scholler 2006; Negrean, Anastasiu 2006; NOBANIS 2007; Desprez-Loustau et al. 2007; Desprez-Loustau 2009, Mułenko et al. 2010). The disease it causes is known from Austria (Rigler-Hager et al. 2003; Kruse 2013), Czech Republic (Dietrich 2005; Müller 2003), Estonia (Põldmaa 1997), Germany (Scholler 1999; Scholler et al. 2010; Kruse 2013); Hungary (Szabo 2002), Finland (Kurkela et al. 1999, Hantula et al. 2009 Lilja et al. 2011), Norway (Gjaerum et al. 2004), Poland (Wołczańska 1999, Piątek et al. 2001; Mułenko et al. 2006, 2008, 2010), Romania (Negrean, Anastasiu 2006), Switzerland (Meier et al. 2003), Turkey (Sert, Sumbul 2005), Italy (Moricca, Maresi 2010), UK (Stringer 2010; Hantula et al. 2012), Ukraine (Tykhonenko 2011) and from both Americas (Dennis 1970; Gallegos, Cummins 1981; Ginns 1986; Buritica, Pardo Cardona 1996; Hernandez, Hennen 2002; Berndt 2004; Hantula et al. 2012).

The main aim of present study was to indicate the occurrence of *Melampsoridium hiratsukanum* on *Alnus* spp. in Lithuania, to discuss its ecology and distribution patterns.

MATERIAL AND METHODS

Samples of the rust infected leaves of *Alnus incana* (L.) Moench and *A. glutinosa* (L.) Gaertn. were collected from various regions of Lithuania during the autumn of 2009-2012. Observation of disease symptoms (Figs 1, 2) and severity continued until late October each year concerned. For comparison morphologically similar rusts, infected leaves of nearby growing *Betula pendula* Roth, which may occur on alder leaves as well,



Figs1-4. Rust fungus *Melampsoridium hiratsukanum* 1-2. Symptoms on upper part of grey alder *Alnus incana* -leaves (photo by J. Kasparavičius). 3. *Melampsoridium hiratsukanum* uredinium (scale bar = 100 μm). 4. Ostiolar cells of uredinium and regularly echinulate urediniospores of *Melampsoridium hiratsukanum* (scale bar = 20 μm).

were collected. Specimens with characteristic rust symptoms collected during present study and all specimens collected earlier by other mycologists available at the herbarium of the Nature Research Centre, Institute of Botany (BILAS), were microscopically examined using the standard microscopic techniques with a Nikon stereo microscope at the magnifications up to 40x and with an Olympus CX 41 microscope at the magnifications up to 400-1000x. The fungus was identified according to morphological characters using (Kuprevich, Tranzschel 1957; Kaneko, Hiratsuka 1981; Roll-Hansen, Roll-Hansen 1981; Hiratsuka et al. 1992; Hantula et al. 2009).

Description and illustrations were made from fresh preparations in distilled water. Dried voucher specimens are preserved in the BILAS Herbarium.

RESULTS AND DISCUSSION

On alders, three morphologically similar *Melampsoridium* rusts may occur: *M. betulinum* (Pers.) Kleb , *M. hiratsukanum* S. Ito ex Hirats. and *M. alni* (Thüm.) Dietel, but only two of them – *M. betulinum* and *M. hiratsukanum* are known in Europe so far (Hantula et al. 2009). In Lithuania, *M. betulinum* is widely spread on birches, but was not yet recorded on alders (Minkevičius, Ignatavičiūtė 1991). Microscopical

Table 1
Comparison of urediniospores of *M. hiratsukanum* and similar rust species occurring on *Alnus* and *Betula* hosts from Baltic countries and Japan

Rust species	Host	Country	Urediniospores length and width range, μm
M. hiratsukanum	*Alnus incana*	Lithuania	22.6-35.0 × 11.5-15.5
M. hiratsukanum	*Alnus glutinosa*	Lithuania	20.4-32.6 × 9.8-16.0
M. hiratsukanum	*Alnus incana*	Finland (Kurkela et al. 1999)	19.2-34.5 × 10.6-19.6
M. hiratsukanum	*Alnus glutinosa*	Finland (Kurkela et al. 1999)	22.6-35.2 × 10.9-16.6
M. hiratsukanum	*Alnus incana*	Estonia (Kurkela et al. 1999)	17.9-30.9 × 10.6-17.3
M. hiratsukanum	*Alnus glutinosa*	Estonia (Kurkela et al. 1999)	20.9-29.2 × 12.3-16.9
M. hiratsukanum	*Alnus hirsuta*	Japan (Hantula et al. 2009)	17.0-32.6 × 8.8-19.7
M. alni	*Alnus crispa*	Japan (Hantula et al. 2009)	24.4-43.3 × 9.1-16.4
M. betulinum	*Betula pendula*	Finland (Kurkela et al. 1999)	26.5-42.1 × 11.6-19.9
M. betulinum	*Betula pendula*	Lithuania	28.0-42.5 × 11.5-18.4
M. betulinum	*Betula pubescens*	Lithuania (Minkevičiūs, Ignatavičiūtė 1991)	(18)26. 2-32.1(50.0) × (10)12.7-13.9(17.0)
M. betulinum	*Betula pubescens*	Finland (Kurkela et al. 1999)	25.4-45.8 × 11.2-19.2
M. betulinum	*Betula pubescens*	Estonia (Kurkela et al. 1999)	24.9-38.5 × 11.9-17.9

examination of Lithuanian material showed that the rust developing on leaves of *Alnus incana* and on leaves of *A. glutinosa* produced regularly echinulate yellow-orange, ovoid to ellipsoidal urediniospores, commonly 24–32 × 12–15 μm in size with 4–6 bizonate germ pores and by size and morphology differs both from native rust *M. betulinum* and other Asian alder rust, *M. alni* (Tab. 1). Following morphological characters of uredinia and urediospores the rust obtained on *Alnus* leaves in Lithuania was identified as *M. hiratsukanum* (Figs 3-4). Uredinia of *M. hiratsukanum* produced longer, resembling sharp spines, ostiolar cells (up to 50 μm), comparing to morphologically similar *M. betulinum*. Uredinia of *M. betulinum* collected during present study were characterized by shorter ostiolar cells (up to 35 μm long) and by smooth, rounded upper end of urediniospores, which were slightly larger in size, commonly about 26–48 × 12–17 μm. *M. alni* is very similar to *M. betulinum* by size of urediniospores and lack of echinulation at the apex but its urediniospores have two germ pores, one at each end of the spore (Hiratsuka 1927; Kuprevich, Tranzschel 1957; Hantula et al. 2009).

During present investigation, *Larix* spp. trees were checked in various parks and forest plantations as well, but aecial stage of *M. hiratsukanum* was not found. It is possible that the fungus can reproduce and spread only by its urediniospores (Hantula, Scholler 2006). Apparently, reduced fungus life cycle restricted to the telial and uredinal host (*Alnus* spp.) prevails in Lithuania.

Examined specimens: *Melampsoridium hiratsukanum*: on *Alnus incana*, Gružu forest, Ukmergė distr., 10 Sep., 1997, leg. A.Treigienė, det. Ignatavičiūtė, BILAS 31446; on *A. incana,* Vilainiai forest Kėdainiai distr., 16 Sep., 1998, leg. A.Treigienė, det. Ignatavičiūtė; BILAS 31445; on *A. incana*, Plateliai forest, Plungė distr., 3 Sep., 2003, leg. A.Treigienė, det. S. Markovskaja, BILAS 48792 on *A. incana*, Semeliškiai environs, Liaukiškiai forest, Trakai distr., 6 Sep., 2005, leg.A. Treigienė, det. S. Markovskaja, BILAS 32914; on *A. incana*, Vidiškiai forest, Ignalina distr., 28 Aug., 2009, leg./det. S. Markovskaja, BILAS 48794; on *A. incana*, Biržai forest, Biržai distr., 27 Sep., 2009, leg./det. S. Markovskaja, BILAS 48795; on *A. incana*, Sirvėtos forest, Švenčionys distr., 29 Aug., 2009, leg./det. S. Markovskaja, BILAS 48797; on

A. incana, Labanoras forest, Molėtai distr., 28 Aug., 2009, leg./det. S. Markovskaja, BILAS 48798; on *A. incana*, Pabradė environs, Švenčionys distr., 29 Aug., 2009, leg./det. S. Markovskaja, BILAS 48796; on *A. incana*, Curonian Spit, Neringa, Smiltynė, 8 Sep. 2009, leg./det. S. Markovskaja, BILAS 48791; *M. hiratsukanum* on *Alnus incana*, Vabalininkų environs, Panevežys distr., 26 Aug., 2009, leg./det. S. Markovskaja, BILAS 49521; on *A. incana*, Petriškiai forest, Širvintai distr., 10 Oct., 2010, leg. A. Treigienė, det. S. Markovskaja, BILAS 48932; on *A. incana*, Jurkiškiai forest, Molėtai distr., 22 Sep., 2011, leg./det. S. Markovskaja, BILAS 50311; on *A. incana*, Akmena environs, Vilnius distr., 23 Sep., 2011, leg./det. S. Markovskaja, BILAS 50309; on *A. incana*, Antaviliai forest, Vilnius distr., 5 Sep., 2011, leg./det. S. Markovskaja, BILAS 50310; on *A. incana*, Curonian Spit, Neringa, Juodkrantė environs, 4 Sep. 2012, leg./det. S. Markovskaja, BILAS 50314; on *A. incana*, shoreline of Curonian Lagoon, Klaipėda distr. 9 Sep. 2012, leg./det. S. Markovskaja, BILAS 50312; on *A. incana*, Aukštadvarys environs, Trakai distr., 11 Oct., 2012, leg./det. S. Markovskaja, BILAS 50316; on *A. incana*, Rudninkai forest, Šalčininkai distr., 21 Aug., 2012, leg./det. S. Markovskaja, BILAS 50315; on *A. incana*, Čepkeliai forest, Varėna distr., 8 Sep., 2012, leg./det. S. Markovskaja, BILAS 50317 on *A. incana*, Kaunas environs, Kaunas distr., 6 Sep., 2012, leg./det. S. Markovskaja, BILAS 50318; on *Alnus glutinosa*, together with *Acalitus brevitarsus* Fockeu, Juodkrantė environs, Neringa, Curonian Spit, 4 Sep. 2010, leg./det. S. Markovskaja, BILAS 48933; on *A. glutinosa*, environs of Lake Tabaliukai, Trakai district, 11 Oct., 2012, leg./det. S. Markovskaja, BILAS 50313; *Melampsoridium betulinum*: on *Betula pendula*, Kretinga environs, Kretinga distr., 27 Aug. 1971, leg. B. Grigaliūnaitė, det. L. Šidla, BILAS 31443; *M. betulinum* on *B. pendula*, Smiltynė forest, Neringa, Curonian Spit, 9 Sep. 2009, leg./det. A. Treigienė, BILAS 48458; *M. betulinum* on *B. pendula* Smiltynė forest, Neringa, Curonian Spit, 8 Sep. 2011, leg./det. S.Markovskaja, BILAS 48459; *M. betulinum* on

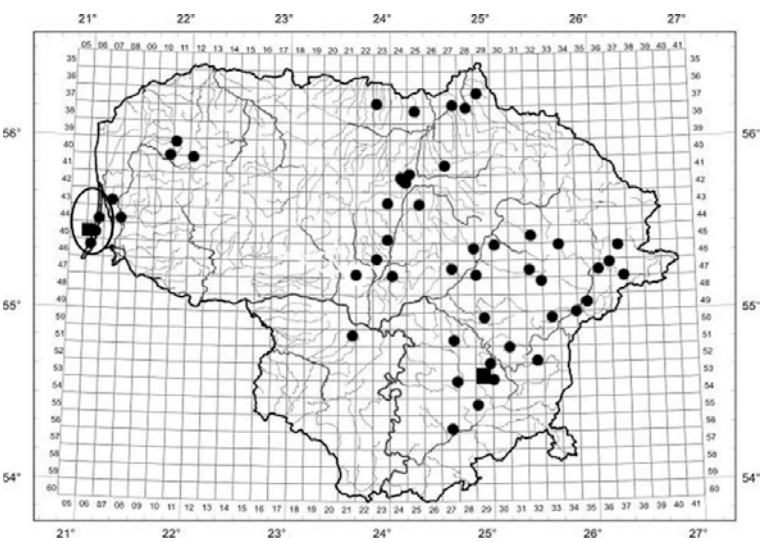
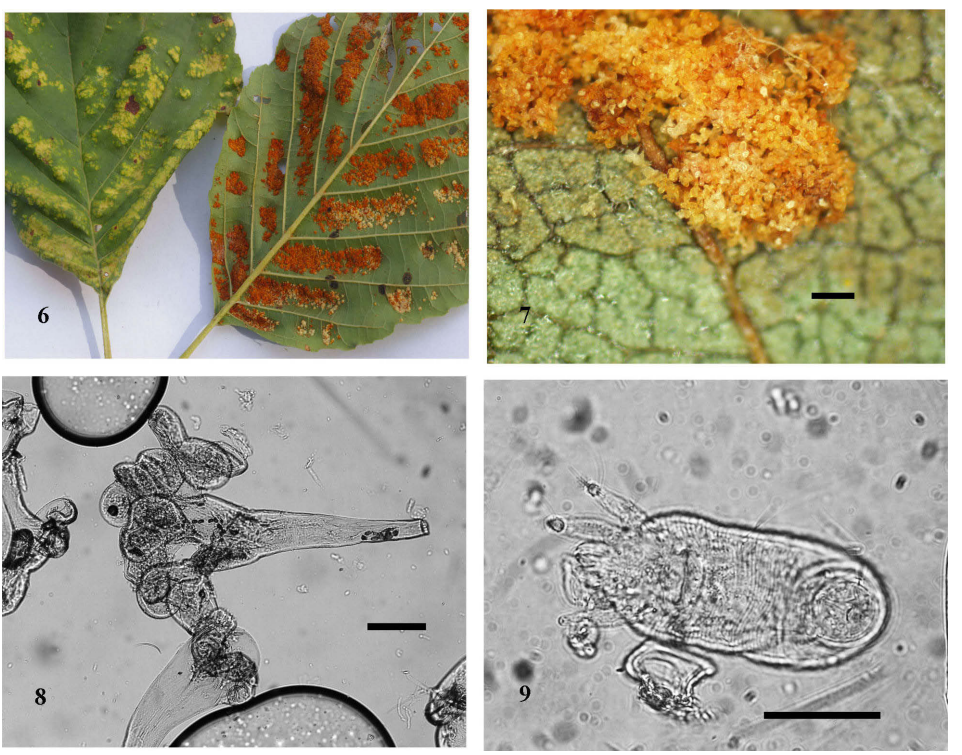


Fig. 5. Distribution map of *Melampsoridium hiratsukanum* in Lithuania (● - on *Alnus incana*, ■ - on *Alnus glutinosa*). The locality of the great cormorant colony (Curonian Spit, area in which *M. hiratsukanum* co-occurred with *Acalitus brevitarsus*) is marked by ellipse.

B. pendula, Juodkrantė environs, Neringa, Curonian Spit, 5 Sep. 2012, leg./det. S. Markovskaja, BILAS 50319.

Distribution in Lithuania. After inspecting mixed forest stands, parks and other habitats in various regions of Lithuania, it was assessed that *M. hiratsukanum* is already widely spread in the country on *Alnus incana* (leaves' infection in some cases reached 20 to 80%) while on *A. glutinosa* it has appeared recently and does not cause strong damage of leaves (about 10%). Most strongly infected (up to 60-80% of leaves) and defoliated already in September were juvenile grey alder trees growing along roads and water bodies. From 2009 till 2012, *M. hiratsukanum* was recorded on *Alnus incana* in 22 localities of Biržai, Kaunas Kėdainiai, Klaipėda, Kretinga, Molėtai, Ignalina, Panevežys, Plungė, Šalčininkai, Širvintai, Švenčionys, Ukmergė, Trakai, and Vilnius districts, while on *Alnus glutinosa* it was found only twice, for the first time in 2010 at the edge of the great cormorant (*Phalacrocorax carbo sinensis*) colony (Curonian Spit, environs of Juodkrantė) and later, in 2012, in the environs of Lake Tabaliukai, Trakai district (Fig. 5).



Figs 6-9. *Acalitus brevitarsus.* 6. Deformation of *Alnus glutinosa* leaves or blister-like galls and erineum (photo by R. Iršėnaitė). 7. Erineum - clusters of hairs on an upper leaf surface (Scale bar = 10 mm, photo by P. Frey from Lithuanian material). 8. An erineum hairs produced by leaf infested by mite (scale bar = 20 μm, photo by P. Frey from Lithuanian material). 9. Microscopic mite *Acalitus brevitarsus* (scale bar = 100 μm, photo by P. Frey from Lithuanian material).

Notable, that in Curonian Spit, in the forest stand affected by great cormorant colony, *M. hiratsukanum* infected leaves of both *A. incana* and *A. glutinosa* trees growing nearby and in some cases *M. hiratsukanum* infected *Alnus glutinosa* leaves together with an alder pest, microscopic mite *Acalitus brevitarsus* Fockeu. Symptoms caused on leaves by *A. brevitarsus* resemble rust fungus infection. This small arthropode, eriophyid mite (*Eriophyidae, Acari, Arthropoda*) inhabit exclusively *Alnus glutinosa* leaves (Figs 6-9). Like many gall-inducing mites, individuals of *A. brevitarsus* cause deformation of leaves or blister-like galls and elicit growth of clusters of hairs on upper surface of leaf (called an erineum) among which the mites live (Figs 6, 7). The hairs have several near-horizontal branches at the tip (Fig. 8), so each group of hairs is like a forest with a closed canopy. Both the pest *A. brevitarsus* and the rust fungus *M. hiratsukanum* were recorded together on the same host leaves only in the stand affected by cormorants (Curonian Spit). In the other parts of Curonian Spit and in the continental part of Lithuania they have never been found to occur together. Evidently, the trees affected by cormorant excrements became less resistant to various pathogens and pests. Successive infections of both agents can cause considerable damage to alder foliage and thus negatively influence the tree health and the functioning of the whole forest ecosystem.

CONCLUSION

Comparison of morphological data indicated that alders and birches in Lithuania are infected by different rust species. Leaves of *Alnus incana* and *A. glutinosa* are damaged by invasive Asian rust *M. hiratsukanum*, while native *M. betulinum* inhabits birch leaves. From its first record in 1997 till now *M. hiratsukanum* has widely spread in Lithuania on *A. incana*. On *A. glutinosa M. hiratsukanum* was recorded for the first time in 2010, co-occurring with eriophyid mite *Acalitus brevitarsus*. It is hypothesized that the successive colonization and co-occurrence of new pathogen (*M. hiratsukanum*) and pest (*Acalitus brevitarsus*) on back alder was a result to the changes (decrease of trees resistance) caused by a strong negative impact of great cormorant colony, namely by forest hypertrophication from the bird excrements.

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