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Cytospora Cankers on Tree Plants in Urban Areas (Karaganda, Astana, Pavlodar) of Central and Northern Kazakhstan

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Abstract

Cytospora canker is a serious fungal disease attacking many types of fruit and ornamental trees in urban parks and gardens. This factor is important when it comes to identifying the disease, as well as to studying its distribution and determining the best methods for its control. The purpose of this article is to define the species composition, host plants, seasonal dynamics of *Cytospora* cankers on tree plants in major cities of Central and Northern Kazakhstan (Karaganda, Astana and Pavlodar). 5 *Cytospora* species were recorded on leaves and bark of the examined trees during the research: *Cytospora leucosperma* (Pers.) Fr**.** on *Betula pendula* L.*,* *Cytospora microspora* (Corda) Rabenh. on *Crataegus sanguinea* Pall., *Cytospora salicis* on *Salix fragilis* L., *Cytospora salicis* (Cda.) Rad. on *Populus nigra* L. and *Cytospora pseudoplatani* Sacc. on *Acer negundo* L. *Cytospora* species development turned to be dependent on the amount of precipitations and was slightly affected by temperature. *Cytospora salicis (Cda.) Rad* on *Populus* nigra L. and *Cytospora leucosperma (Pers.) Fr* on *Betula pendula* L. were new species for Kazakhstan. *Cytospora salicis* (Cda.) Rad, that parasitizes on the Populus nigra L. appeared the most hazardous specie.

Keywords: *Cytospora* cankers, host plants, fungal disease, stroma, endophytic pathogens

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INTRODUCTION

Cytospora Ehrenb. genus, which was described by Ehrenberg in 1818, causes ulcers and death of plant parts in many genera of deciduous and coniferous trees, but rarely in grassy plants. A number of authors (Farr et al. 1989, Sinclair et al. 19867) listed more than 85 species of woody plants susceptible to Cytospora ulcers, sometimes called *Valsa* canker, *Leucostoma* canker or *Perennial* canker. Cytospora ulcers are characterized by the presence of diffusely-spread resin on branches with fruit organs. The lower branches are first affected, after that the ulcers gradually rise upward (Mehrabi et al. 2011). Pathogens infect the inner bark, or periderm of the bark. In the deciduous forests, the cork (phloem) adjacent to the periderm is discolored. Coniferous trees have no discoloration, although the fungus can recover from xylem (Schoeneweiss 1983). In the affected wood, hydraulic conductivity of the xylem vessels is disturbed (Chang 1991), causing the infected branches or trees to wilt and die. *Cytospora* cankers are particularly destructive on *Prunus* spp. in commercial gardens, as well as *Picea* spp. and *Acer* spp. in city parks and on *Populus* spp. in forest units. Interesting fact, that *Cytospora* spp. have been isolated from healthy tissues of many tree species, such as xylem, bark and leaves (Bills 1996), so they may be regarded as endophytes in xylem, bark and leaves (Fisher 1993). At the same time, some other species can be strictly saprobic on dying trees (Christensen 1940).

Fungi of the genus Cytospora are included in *Sphaeropsidales* order and are an anamorphic stage of

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development of such genera as *Valsa* Fr., *Leucocytospora*, *Leucostoma* (Nitschke.) Höhn, *Valsella* Fuckel and *Valseutypella* Höhn (Adams et al. 2006, Hyde et al. 2016). Therefore, all of these sexual genera were synonymized under *Valsa*, either as a subgenus or species (Adams et al. 2006). Fruit bodies consist of stromateis (conidiomata), which usually contain either labyrinth chambers or pycnidia clusters with thread conidiophores and allantoid hyaline conidia. In wet conditions, conidia are secreted from fruit bodies in gelatinous matrices, usually in the form of yellow, orange, red or pale tendrils (Adams et al. 2006). White, usually angular, spots with brown edges appear on plant leaves due to the influence of the Sphaeropsidales. Initially, the spots are smooth, later they protrude and turn into convex spots (Burova 1986, Cheremisinov 1973). The Sphaeropsidales occur at all stages of the vegetative season. In summer, they appear on plants in the form of spots different in color and shape. They usually affects various parts of plants (Burova 1986, Cheremisinov 1973).

Pathogenicity of the Cytospora can be species-specific, but it is difficult to determine (Adams et al. 2006). For example, Kepley et al. showed that species of *Cytospora* from green ash *(Fraxinus pennsylvanica) (Cytospora pruinosa)*, alder *(Alnus spp.) (Cytospora umbrina*), Siberian elm *(Ulmus pumila) (Cytospora sacculus)*, green aspen (*Populus tremuloides) (Cytospora chrysosperma [Valsa sordida])* and eastern cottonwood (*P. deltoids) (Cytospora chrysosperma)* were host-specific, while isolates from multi-stemmed willow *(Salix spp.) (Cytospora fugax)* were pathogenic for aspen as well (Kepley and Jacobi 2000).

Woody and ornamental flowering plants growing in urban areas should be protected from various diseases and pests. The process of determining species composition, specifics of origin and distribution, and the infectious degree of hazardous organisms on plant species is of great importance (Ainsworth and Sampson 1950, Bisby 1933, Gvritishvili 1969, Parmasto 1968, Saccardo 1882-1931, Transhel 1939, Zhuravlyov 1979, Dudka et al. 1982). *Cytospora* species of Central and Northern Kazakhstan have not been extensively discovered, while awareness of the existing pathogenies fungi species is essential for effective disease management.

The purpose of this research was to define the species composition, host plants, seasonal dynamics of *Cytospora* cankers on tree plants in major cities of Central and Northern Kazakhstan (Karaganda, Astana and Pavlodar). Thereby, we intend to increase the understanding of geographical and host range relationships.

MATERIALS AND METHODS

The subject of the study was the parasitic fungi of the genus *Cytospora*, which were obtained from the damaged dead branches of sick trees, living leaves of trees and shrubs collected during the research in the cities of Astana, Karaganda, Pavlodar, Aksu and Ekibastuz in 2014-2016. Research on changes of the tissue affected by the diseases was carried out by the differential staining method according to the S.I. Vanin's method (1934). Phytopathogenic fungi were determined based on fruit bodies, appendages, asci and spores according to I.I. Zhuravlyov's method (1979). In the course of the study, the MBR-3 microscopes and MBS-1 macroscopic binoculars were used. Yu.V. Sinadsky's method (1964) was used to determine the level of plants infestation, depending on the type of disease, using a 6-point scale. Artificial nutrient media, wet chambers and a thermostat were used in the study of partially obligate parasites,.

To determine the relationship between the seasonal dynamics of Cytospora development and environmental factors such as the monthly mean daytime temperature and the monthly average amount of precipitation we used the Pavlodar hydrometeorology data for April-October 2016.

RESULTS

The study was carried out in Central and Northern Kazakhstan (Karaganda, Astana, Pavlodar) between 2014 and 2016. We have comparatively analyzed the degree of plant damage caused by cankers of pathogenic fungi.

Identification was based on morphological characteristics, including shape, color and size of stroma, number and shape of chambers, shape and size of perithecia and spore characteristics.

In general, 22 isolates from 5 plant species were studied during our research. Based on the morphological features of *Cytospora* isolates, following species were identified: *Cytospora leucosperma (Pers.)* Fr., *Cytospora microspora* (Corda.) Rabenh., *Cytospora salicis, Cytospora salicis (Cda.)* Rad. and *Cytospora pseudoplatani* Sacc.

Cytospora leucosperma (Pers.) Fr. (Fig. 1).

The stromas are scattered or folded, black, 1-2 mm in diameter, multichambered. The chambers are regular

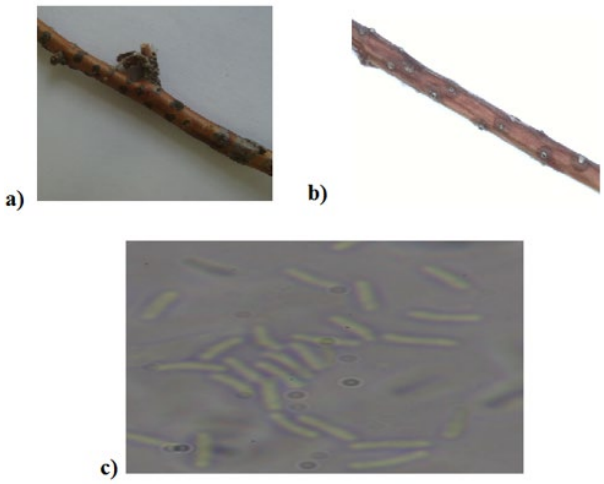

Fig. 1. A – General view of *Betula pendula* L. infested shoot; B – Close view; C – *Cytospora leucosperma (Pers.)* Fr. (conidia) on the shoot

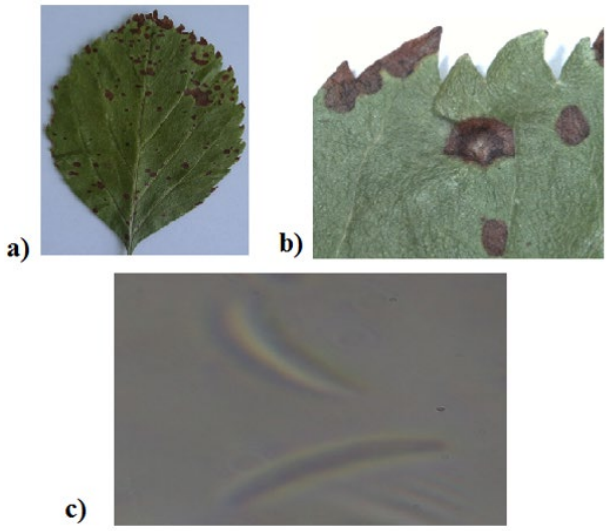

Fig. 2. A - General view of the leaf infected with *Crataegus sanguinea* Pall. ; B –Close view; C – Stylospores of *Cytospora microspora* (Corda) Rabenh on the leaves

radially arranged, round and ovoid. Perithecia are spherical, 256-320 μm. Conidia are colorless, unicellular, hyaline, allantoid, aseptate, 3.5-5.5(4.5) × 0.9-1.2(1.1) μm, issuing in reddish tendrils (Flora sporovykh rastenii Kazakhstana 1961, 1981, Mehrabi et al. 2011).

Host plant: *Betula pendula* L., found on branches.

Specimens were found in Astana, left bank of the Yessil River, 04.07.15, A.D. Spanbayev, Pavlodar, Satpayev Street, 22.08.2015, A.K. Ospanova.

***Cytospora microspora* (Corda) Rabenh. (Fig. 2)**

Stromas are multichambered, 1000-1500 μm, 4-6 chambers, brown. Stylospores are unicellular, 3.3-4.4 x 1.5 μm.

Fig. 3. *Cytospora salicis* on the leaves of *Salix fragilis* L.

Host plant - *Crataegus sanguinea* Pall, found on leaves

Specimens were found in Karaganda, Botanical Garden, Yerzhanov Street, 13.07.2014, A.D. Spanbayev, Pavlodar, Kutuzov Street, 10.08.2015, A.K. Ospanova.

***Cytospora salicis* (Fig. 3)**

Stromas are of elongated shape, 350-550 μm. The color of the ectostroma is pale yellow. No difference in color between endo- and ectostroma is observed.

Perithecia are spherical, 210-340 μm. Clavate sac, 40 – 52.5 x 7 μm, no legs. 6-spored, 9.5-14 x 2-2.5 μm.

Host plant- *Salix fragilis* L., found on leaves.

Specimens were found in Karaganda, Yerubaev Street, 03.09.16, A.D. Spanbayev. Pavlodar, Central Park, 15.08.16, A.K. Ospanova.

***Cytospora salicis* (Cda.) Rad. (Fig. 4)**

Stromas are elongated, 350-550 μm. Color of the extra stroma is pale yellow. No difference in color between the endo- and ectostroma is observed.

Fig. 4. *Cytospora salicis* (Cda.) Rad. on the shoot of *Populus nigra* L.

Fig. 5. *Cytospora pseudoplatani* Sacc. on the shoot of *Acer negundo* L.

Perithecia are spherical, 220-380 μm. The sac is clavate, 40 – 52.5 x 7 μm, no legs, 8-spored, 10.5-14 x 2-2.5 μm.

Host plant – *Populus nigra L.*, found on shoots.

Specimens were found in Pavlodar, Lomov Street, 24.09.2016, Ekibastuz, Pobeda recreation area, 29.09.2016, A.K. Ospanova. Karaganda, Central Park, 16.07.2016, A.D. Spanbayev. Astana, Central Park, 16.07.2016, A.D. Spanbayev.

***Cytospora pseudoplatani* Sacc. (Fig. 5)**

Stromas are scattered or folded, black, multichambered.

The chamber is round and ovate. The wall of the chamber is thick, 12-20 μm. The conidia are colorless, unicellular, 3-7 x 1.5 μm.

Host plant - *Acer negundo* L., found on shoots. New host plant for Kazakhstan.

Specimens were found in Pavlodar, Chokin Street, 29.09.2014, A.K. Ospanova.

Table 1 shows the degree of plants infestation on a 6-point scale. These indicators help to see, which plants and in which cities were infected and the extent to which the plants were infected.

Table 1. The degree of plants infection with phytopathogenic fungi

Plant	*Cytospora* species	Degree of infection (grades 1-6)		
		Astana	Karaganda	Pavlodar
1. *Betula pendula* L.	*1.Cytospora leucosperma* (Pers.) Fr.	*4*	-	3
2.*Crataegus sanguinea* Pall.	*2.Cytospora microspora* (Corda) Rabenh.	-	*4*	4
3. *Salix fragilis* L	*3. Cytospora salicis*	-	3	3
4.*Populus nigra* L.	*4. Cytospora salicis* (Cda.) Rad.	5	6	6
5.Acer negundo L.	5. *Cytospora pseudopla tani* Sacc.	-	-	4

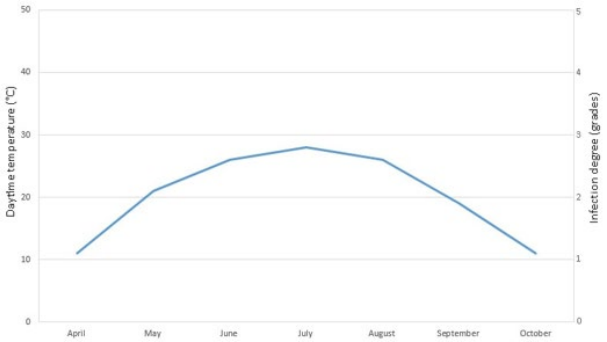

Fig. 6. Dynamics of *Cytospora spp.* development depending on the average daytime temperature in Pavlodar in 2016

Fig. 7. Dynamics of *Cytospora spp.* development depending on the amount of precipitation in Pavlodar in 2016

According to the base of identified specimens collected in this region, *Cytospora salicis* (Cda.) Rad. was the dominant and the most hazardous specie for black poplar.

Also during our research, we investigated the relations between *Cytospora* spp. development and weather characteristics. **Figs. 6** and **7** show the abundant distribution and development of *Cytospora* spp. in July-August 2016 on the example of Pavlodar. The figures reflect the direct dependence of the average daytime temperature or amount of precipitation and degree of trees infection.

DISCUSSIONS

Since 1980s, after publication of such works as by Redlin and Carris (1996) the attention has turned to an increased realization of the endophytic and latent nature of many leaf and stem pathogens pests. Later on, Adams et al. confirmed that except some species of *Cytospora*, that course infection after invasion through wounds, there are species that canker healthy trees, causing latent infection and disease following some stimulus (Adams et al. 2005).

The list of the most important recent monographic works related to *Cytospora* species includes the works by Kobayashi (1970) devoted to species in Japan, Gvritishvili (1982) and Vasilyeva (1994) devoted to species in the former Union of Soviet Socialist Republics (USSR), Spielman (1983, 1985) devoted to North American species on hardwoods, and Adams et al. (2005) devoted to species on Eucalyptus (Adams et al. 2006).

Cytospora canker is a serious fungal disease that attacks many shade, fruit, and ornamental tree species. This is why the need for understanding specific features of its development does not rise doubt. During current research, we showed that *Cytospora spp.* is slightly affected by temperature changes, but it needs a lot of precipitation. James Worrall et al. during the study of *Cytospora* canker of Alnus revealed that canker expansion and death of branches and stems occurred almost exclusively in the warmest time of summer (Worrall et al. 2010. Moreover, his research was conducted in the conditions of monsoon climate, which means that humidity in summer is much higher than in other seasons. On the other hand, the most of tree *Cytospora* spp. are considered as wound parasites that attack weakened trees (Adams et al. 2006), that is why low temperature predisposes trees for *Cytospora* cancers, causing buds and dormant twigs injuring (Reich and van der Kamp 1993).

Based on our findings, *Cytospora* species could be arranged in the following row depending on their pathogenicity: *Cytospora salicis (Cda.) Rad*> *Cytospora microspora (Corda) Rabenh.*> *Cytospora leucosperma (Pers.) Fr.*> *Cytospora pseudoplatani Sacc.*> *Cytospora salicis.*

During another study, conducted in woody-bush plantations of the Pavlodar region of Kazakhstan *Cytospora salicis* was also found on *Salix alba* in Pavlodar and Ekibastuz cities. It had maximum degree of infection at the 3-point scale, while *Cytospora pseudoplatani*, that was found on *Acer negundo* in Pavlodar had minimum degree of infection (Ospanova 2006). In our study, *Populus nigra* appeared to be the most affected species, followed by *Salix alba, Pinus sylvestris and Caragana arborescens* (Ospanova 2006). We have analyzed the *Cytospora* species of Kazakhstan and found *Cytospora microspora* (Cda.) Rab. along with other 180 microfungi species in fruit forests of Ili-Alatau National Park on *Sorbus tianschanica and Crataegus spp* (Rakhimova et al. 2005). During the current study, such species as *Cytospora salicis (Cda.) Rad* and *Cytospora leucosperma (Pers.) Fr.* were shown to infect the tree plants in the urban areas of Central and Northern Kazakhstan for the first time.

Based on the research findings, we can state that *Cytospora salicis* (Cda.) Rad is the most hazardous species of the fungus for the black poplar. Kepley and Jacobi also showed that high virulence and destruction *Cytospora* spp. reveals with respect to the Populus species (Kepley and Jacobi 2000). The same is true for *Prunus spp* (Biggs 1989). Since the black poplar (*Populus nigra* L.) is annually affected with the cytosporosis (pathogen – *Cytospora salicis* Rad.) in Pavlodar, we have developed measures to fight with the disease. They were undertaken during the vegetative season of plant development. We used 3 preparations: 0.5% Zineb solution, 0.3% Bayleton solution and 1% Homa solution. Treatment was carried out 3 times with a periodicity of 7 days. The most effective was the 0.3% aqueous bayleton solution. Isin and Tuleuov proposed another method of Cytospora control – 20% emulsion of copper naphthenate was applied 2-3 times in early spring at 10-15-day intervals (Isin and Tuleuov 1970).

To protect plants and reduce the damage caused by *Cytospora spp.* in the regions of Central and Northern Kazakhstan, there should be more researches on pathogenicity and physiology of this fungal group.

CONCLUSIONS

Cytospora salicis (Cda.) Rad and *Cytospora leucosperma (Pers.) Fr*, that were found on the *Populus* nigra L. and *Betula pendula* L. respectively, were shown to infect tree plants in the urban areas of Central and Northern Kazakhstan for the first time. *Acer negundo L.* was shown to be a new host plant for *Cytospora pseudoplatani* Sacc. in Kazakhstan. *Cytospora salicis* (Cda.) Rad appeared to be the most hazardous species of fungus for the black poplar (*Populus nigra* L.). *Cytospora* spp. is slightly affected by temperature changes, but needs a lot of precipitation.

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