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ORIGINAL ARTICLE

Mildew of oleaster (*Elaeagnus oxycarpa* Schlecht.) registered in large industrial cities (Pavlodar, Aksu, Ekibastuz) of the Pavlodar region



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Abstract *Purpose:* Vegetation plays a very important role in industrial regions in terms of not only air purification, but also oxygen enrichment, air humidity, and city aesthetics. The paper presents the authors' study of oleaster mildew during 2012–2013 in the cities of Pavlodar, Ekibastuz, and Aksu. *Method:* The species composition of mildew, patterns of its growth and reproduction, seasonal dynamics, and the level of trees infection according to a six-score scale were determined. *Result:* Three cultivars of mildew from three genera were registered. The species composition of mildew of oleaster is studied for the first time in large industrial cities of the Pavlodar region. The comparative analysis of the systematic structure of phytopathogenic fungi was followed by the study of the micro flora of green planting in megalopolises. The seasonal dynamics of growth, development, and reproduction was determined. The impact of pests on host-plants was studied. *Conclusion:* Timely diagnosis and localization of disease outbreak increases the likelihood of successful treatment and saving of plants.

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1. Introduction

Vegetation plays a very important role in industrial regions in terms of not only purification of air by removing various harmful substances, but also oxygen enrichment, increase in air humidity during hot summer days, noise reduction, and city aesthetics in general.

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At present, there are strict environmental requirements to the design of the structure of populated places, megalopolises in particular. The development of scientifically substantiated works on greenery planting is a necessary stage that demands close attention.

In order for various trees, bushes, and decorative flowering plants to serve humanity for a long time, they need to be not only adequately taken care of, but also protected from various plant diseases and blights. Therefore, it is necessary to determine the species composition, peculiarities of origin and reproduction, and the level of infection rate by hazardous organisms with regard to the cultivar, age, and habitat.

1.1. *Erysiphales* order

Powdery mildews are one of the most easily recognizable worldwide plant diseases. They are characterized by unsightly, white or grey powdery growths of fungal mycelium and/or spores on leaves and stems and sometimes on flowers and fruits. These symptoms, though similar in appearance, are caused by different powdery mildew fungi in the *Erysiphales* order, which are considered obligate parasites, since they require a living host to survive (Marçais and Desprez-Loustau, 2014).

All attempts to grow them on artificial media have failed thus far. They are also host-specific or restricted to a limited range of host-plants. However, some mildews can spread to cultivated plants from closely related weed hosts. The disease develops best in a dry climate with a relative humidity of 79–99% and temperatures around 15–27 °C, which are common conditions in a cool, dry, and misty season from November to February (Tello et al., 2005).

In terms of pathogeny, phylogeny, taxonomy, and nomenclature, based on 18S rRNA gene sequences, powdery mildews are placed together with inoperculate discomycetes in the Leotiomyces class, regardless of morphological differences (Saenz et al., 1994). Their molecular phylogeny and morphologic taxonomy are quite settled at present with six clades and five tribes complementing or corresponding with each other (Saenz and Taylor, 1999; Braun and Takamatsu, 2000; Braun, 2002). Sequence analyses of the polymerase chain reaction in the amplified internal transcribed spacer region of rDNA is commonly employed in molecular studies of powdery mildews (Weis et al., 2013).

Conidial morphology and development, location of mycelium, and host associations are characteristics that help define and classify anamorphs. Careful observations and interpretations of structures using light and scanning electron microscopy (Cook et al., 1997) or brightfield microscopy and differential interference contrast microscopy (Glawe, 2008) can help minimize subsequent revisions. In teleomorphs, large-sized chasmothecia, containing many 8-spored asci, had many appendages with uncinat-circinate apices, arising around the supraequatorial part of chasmothecia. In anamorphs, conidia were produced in chains (Euoidium-type) without distinct fibrosin bodies (Takamatsu, 2013).

1.2. Taxonomy

At present powdery mildews are defined and/or recorded; they are classified and listed below in alphabetical order together

with the host, location, taxonomic notes, and intercontinental distribution data.

Division ASCOMYCOTA (Whittaker, 1959).

Class LEOTIOMYCETES.

Subclass LEOTIOMYCETIDAE. (ERYSIPHOMYCETIDAE).

Order ERYSIPHALES (Gwynne-Vaughan, 1922).

Family ERYSIPHACEAE (Barr, 2009).

In most cases, pathogen effectors prevent recognition or suppress host defence. However, successful suppression of host defence is not always sufficient for pathogenesis, which requires further host components that meet the demands of pathogen development and nutrition (Hückelhoven et al., 2013). The mycelia of powdery mildews usually occur on the leaves, small stems or twigs of seed plants; those, who are accustomed to observing small fungi, if the mycelia are sufficiently conspicuous, easily collect these fungi. In summer and autumn, these parasites may be observed on the leaves of goldenrods, asters, sunflowers, yard grass, ragweed, verbenas, roses, willows, oaks, lilacs, and other herbs, shrubs, and trees.

Less conspicuous mycelia occur on yellow sorrel, grapes, hackberry, tulip poplar, Ohio buckeye, maples, elms, chestnut, and other seed plants. In order to know whether the perithecia are in good condition and to see the inconspicuous forms at all, one should collect with a hand lens at hand. Preliminary reference to a host index will significantly improve the success of the collecting trip.

The Pavlodar region is a big industrial area in North-Eastern Kazakhstan. There are several large factories in Pavlodar city itself. These include electrolysis and aluminium plants, as well as oil refineries. There is an open coal deposit near Ekibastuz city, which supplies three operating fossil fuel burning power stations. Aksu city is in similar environmental conditions.

This paper presents the results of a study of vegetation diseases in large industrial cities of the region, whose environmental situation is a cause of concern.

Research purpose and objectives:

- to analyse comprehensively the species composition and distribution patterns of mildew of oleaster (*Elaeagnus oxycarpa* Schlecht), which grows in big industrial cities of the Pavlodar region;
- to determine the species composition of phytopathogenic mildew of oleaster (*E. oxycarpa* Schlecht.);
- to analyse the systematic structure;
- to study seasonal dynamics of fungi.

2. Materials and methods

The research materials included parasitic fungi of trees, herbaria with infected organs, as well as their fruit bodies and spores, collected by the authors in the territory of Pavlodar (52°18'N 76°57'E), Ekibastuz (51°40'0"N 75°22'0"E) and Aksu (52°02'0"N 76°55'0"E) in 2012–2013.

In addition to common mycological methods, the collection and study of phytopathogens drew upon the works and guides of Ainsworth and Sampson, 1950. When defining other cultivars, the authors used the guides of Parmasto (1970).

Zhuravlev's method (1979) was used for revealing and defining. Sinadskii's six-score scale (Sinadskiy, 1964) was used to determine the type and level of plant infection.

Seasonal dynamics of phytopathogenic mildew and its annual level of development were studied, based on weather data and precipitation amount. All necessary data were obtained from meteorological stations of Pavlodar, Ekibastuz, and Aksu.

The ordinal species composition of mildew of oleaster (*E. oxycarpa* Schlecht) is presented below (Table 1).

Freshly collected specimens were directly examined in situ with a binocular dissecting microscope (Omano OM-20-1L, Japan) for gross macrocharacteristics. Then they were examined under a light microscope (Olympus BX40, Japan) while mounted on slides in 85% lactic acid with aniline blue. Fully developed spores were measured to obtain a median of the spore range.

Definitions were made, based on anamorphic and/or teleomorphic characteristics. All references cited herein (Sivanesan, 1976; Hanlin, 1998) were consulted for description, identification, distribution, nomenclature, comparison, earlier records, etc. and individually mentioned in the text for reference, where necessary.

Specimens deposited in part at Herb. IMI, UC (= LAM) and the Department of Plant Pathology (formerly the Institute of Agriculture, Mandalay), are also included. Herbarium acronyms are taken from the Index Herbariorum database ("<http://sciweb.nybg.org/science2/IndexHerbariorum.asp>," n.d.); plant names from the IPNI database ("<http://www.ipni.org/ipni/plantnamesearchpage.do>," n.d.), standardized in accordance with (Brummitt and Powell, 1992); fungus names from the CBS database ("<http://www.cbs.knaw.nl/databases>," n.d.) and the Index Fungorum database ("<http://www.indexfungorum.org/Names/NAMES.ASP>," n.d.), and fungus-host distribution from the SBML database ("<http://nt.ars-grin.gov/fungaldatabases/fungushost/FungusHost.cfm>," n.d.).

After each citation, the year of publication is given, if available. Names of old records after updating and integration are retained enclosed after the current, accepted epithets, to aid in referencing. Vernacular names, although not specific, but sometimes descriptive, are provided when available to fill in gaps in the host nomenclature.

3. Results

Powdery mildews should be collected as soon as the perithecia mature and, if possible, while the mycelium is still conspicuous. Of course, this combination is impossible in species with very evanescent mycelia. Finally, the collecting should be done

before wind and rain have caused nearly all the mature perithecia to disappear or these fruits have become so ripe that they fall off shortly after being collected.

More than once, such material, collected in quantity for laboratory studies, has proven satisfactory soon after being brought in from the field, but has been found so devoid of perithecia by the next year as to be practically useless for any purpose. For a successful study, the conidial stage must be taken in its prime, i.e. before the perithecia mature.

Young mycelia with such conidial conditions may be found at any time from the middle of June until late autumn. While looking for silvery-white, often glistening stages, devoid of perithecia, any species with a reasonably conspicuous mycelium will serve for this purpose. *Erysiphe graminis* on grasses, the species on roses, and some forms on plaintain are likely to give good conidial conditions in the late season, while similarly good material may be found on asters and other hosts.

3.1. Genus *Leveillula*

3.1.1. *Leveillula taurica* Arnaud f. *elaeagni* Jacz. (*Leveillula elaeagni* (Jacz.) (Braun, 1987))

Found on the abaxial surface of an oleaster leaf. A lot of cleistocium, capacity is 180–200 micron. Appendages are well developed, many pouches. Six spores per pouch. Spores are oval; volume is 20.9–27 × 15.2–19 µm.

3.1.2. Host-plant

E. oxycarpa Schlecht., were found on leaves.

3.1.3. Location in Kazakhstan

Aksu city, Stroitel'naya Street. July 2012–2013, A.K. Ospanova.

3.2. Genus *Uncinula*

3.2.1. *Uncinula* sp.

On the entire surface of oleaster leaves in the form of minute spots in great numbers. A lot of cleistocium, capacity is 140–158 µm. Number of pouches 4–5, capacity 18–205 micron. Spore count 3–5; volume is 12–18 × 6–8 µm.

3.2.2. Host-plant

E. oxycarpa Schlecht., were found on leaves.

3.2.3. Location in Kazakhstan

Ekibastuz city, Central Park, 28.09.2013, A.K. Ospanova. Figs. 1 and 2 show a new host-plant and a new species for Kazakhstan.

3.3. Genus *Phyllactinia*

3.3.1. *Phyllactinia suffulta* Sacc. f. *elaeagni* Jacz.

Conidia resemble pins. Cleistocia are spherical, volume 168–256 micron. Many pouches. Spore count 2; volume is 26–30 × 10–15 µm.

3.3.2. Host-plant

E. oxycarpa Schlecht., were found on leaves.

Table 1 Order *Erysiphales* taxonomic species composition of mildew of oleaster.

| Genus | Number of species | | |
|---------------------|-------------------|------|-----------|
| | Pavlodar | Aksu | Ekibastuz |
| 1 | 2 | 3 | 4 |
| <i>Leveillula</i> | 1 | 1 | 1 |
| <i>Uncinula</i> | 2 | 2 | 1 |
| <i>Phyllactinia</i> | 4 | 2 | 4 |



Figure 1 *Uncinula* sp. found on leaves of *Elaeagnus oxycarpa* Schlecht.

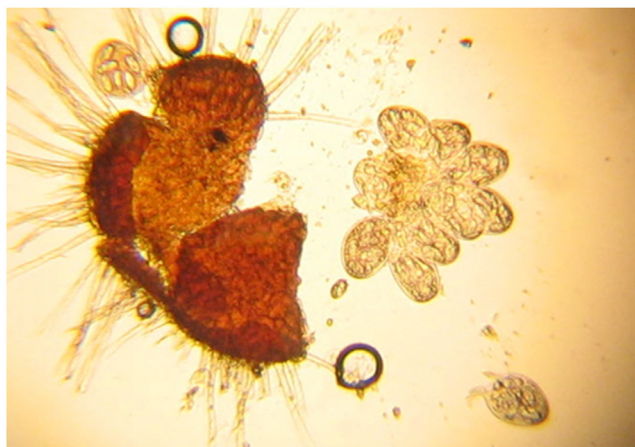


Figure 2 Appendages, cleistothecium, pouches, spores of mildew *Uncinula* sp.

3.3.3. Location in Kazakhstan

Pavlodar city, Satpaev Street, 22.08.2013, A.K. Ospanova. A new host-plant for Kazakhstan.

Table 2 shows the degree of plant infection according to a six-score scale. These indicators display the level of tree infection in each city.

The results showed that the seasonal dynamics of mildew in the three cities were similar. They affect various decorative trees, shrubs and flowering plants. The abaxial surfaces of leaves have mycelia and conidia of mildew. Infected trees grow and develop slowly and have worse decorative features.

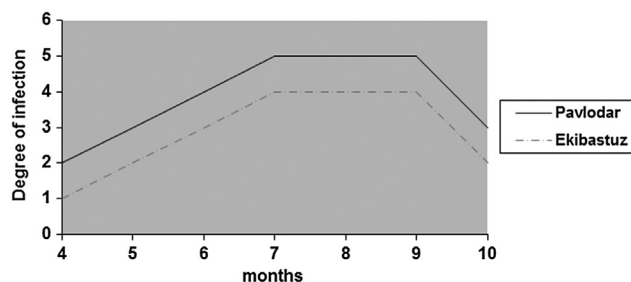


Figure 3 Dynamics of development of mildew in 2012–2013.

In the studied cities, the seasonal dynamics of mildew begin in June–July with the conidial stage and come to the stage of cleistothecia ripening in August–September (Fig. 3). While wintering in plant residues, adapted fungi form different kinds of fruit bodies, which crack in spring and release spores. In 2012–2013, there were favourable conditions in the three cities for the development and growth of mildew (high air temperature and low humidity).

4. Discussion

E. oxycarpa is important in the flora of Central Asia. As halophytes, its growth pattern does not change in the presence of low salinity (da Silva et al., 2008). It can be grown in up to 300 mM NaCl without any effect on survival (Maimaiti et al., 2014). The plant carries positive low values of soil moisture (Murata et al., 2012). It carries well the urban load, dust and exhaust gases. Thus, *E. Oxycarpa* is ideal for distribution in Kazakhstan; the edible fruits are widely used as food. The most dangerous and common pathology is a powder mildew fungi. These parasitic ascomycetes tend to grow superficially, or epiphytically, on plant surfaces. Kazakhstan fungi occurring were studied and it showed that mildew is widespread for all Rosaceae (Valiyeva et al., 2005). However, the previous studies did not include the monitoring of *E. oxycarpa*. In this article, we focus on powder mildew of urban spaces. Plants that grow in urban conditions, usually have a decorative function. The affected units stop in development, seriously suffer the humidity extremes and usually are removed from the plantations. A mechanism, by which the differential severity of disease on upper and lower leaf surfaces can be explained, is based upon ontogenic resistance, leaf folding, and escape from infections. A high degree of leaf folding in the earliest developmental stages is directly responsible for the absence of disease on the upper leaf surfaces when leaves are exposed to airborne conidia (Asalf et al., 2014).

Real-Time PCR was used in order to diagnose the disease, what along with the partial sequences of the internal tran-

Table 2 Level of mildew infection of oleaster in some cities.

| Fungi species | Level of infection | Pavlodar | Ekibastuz | Aksu |
|--|--------------------|----------|-----------|------|
| 2 | 3 | 4 | 5 | 6 |
| <i>Leveillula taurica</i> Arnaud f. <i>elaegni</i> Jacz. | 3 | 3 | – | 2 |
| <i>Uncinula</i> sp. | 4 | – | 4 | – |
| <i>Phyllactinia suffulta</i> Sacc. f. <i>elaegni</i> Jacz. | 5 | 5 | – | – |

scribed spacer of ribosomal DNA (ITS) using provides a quick and accurate identification of pathogens without the risk of contamination of the work area with amplification products (Beyshova et al., 2015). This method showed good results in the study of powdery mildew on *Arabidopsis thaliana* (WeBling and Panstruga, 2012). However, for practical usage of the state control services, this method is not optimal. High prices of equipment and materials limit the widespread identification. Visual identification of the pathogen is a simpler and practical method, the equipment can be adapted to mobile laboratories. With emergence of online databases, gathering the required information is becoming easier and cheaper. As online databases continue to improve and the new analytical instruments are developed, this approach will become even more powerful (Lavoie et al., 2013). The taxonomic name of an organism is a key link between different databases that store information on that organism. However, in the absence of a single, comprehensive database of organism names, individual databases lack of the easy means of checking the correctness of a name. Furthermore, the same organism may have more than one name, and the same name may apply to more than one organism (Page, 2005). Web services approach to providing access to taxonomic names. Using the virtual databases in modern ecology is a wider range of applications. One of the main problems is the lack of common standards for the construction of these systems. Each of them has its advantages and disadvantages. Index Fungorum database can be sourced to find taxonomic details about fungi, while DNA sequence data can be sourced from NCBI, EBI and UNITE databases. Although the sequence data may be linked to a name, the quality of the metadata is variable and generally there is no corresponding link to images, descriptions or herbarium material (Jayasiri et al., 2015). SBML is regarded as a de facto standard to express the biological network data in biology systems, the amount of the SBML documents is exponentially increasing. For high quality data, SMS supports data cleansing function using gene ontology (Jung et al., 2006).

Timely diagnosis and localization of disease outbreak increases the likelihood of successful treatment and saving of the plant (Twomey et al., 2015). The global trade in 'plants for planting' is a recognized pathway for the accidental introduction of pests and pathogens, even though the plant health legislation exists to minimize such accidental introductions (Tubby and Webber, 2010). Creation of the national programme on liquidation of the foci of fungi infection will help to minimize the loss of resources.

5. Conclusions

Thanks to its unpretentiousness in growing conditions, the oleaster (*E. oxycarpa* Schlecht.) is a widespread plant in Kazakhstan, including cities. Fungal infection is the greatest danger for the plant. Mildew causes the deterioration of the living plant characteristics, inhibits growth and deteriorates decorative properties. The microbiological monitoring of Pavlodar city park areas, Ekibastuz, and Aksu was conducted. It was defined that mildew of leaves has caused by the pathogens of genus *Leveillula*, *Uncinula*, *Phyllactinia*. High air temperature and low humidity are favourable conditions in the three cities for the development and growth of mildew. Timely detection of the pathogen allows minimizing the economic losses.

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