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PUCCINIOSTELE MANDSCHURICA, DISEASE AGENT OF RUST INFECTION IN *ASTILBE* CULTIVARS

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The rust fungus *Pucciniostele mandschurica* was found on astilbe cultivars in the Botanical Garden-Institute, FEB RAS. The spread of the rust pathogen is characterized as enphytotic. The symptoms of plant infection by *P. mandschurica* are described (decrease in plant height and foliage density; deformation of inflorescences, pustules, galls, yellow spots on the leaves and flower stalks). The condition of astilbe in the collection is evaluated (as excellent, good, medium, or bad) depending on manifestation of signs of disease. To calculate the intensity of infection development (R, %), an original 5-point (from 0 to 4) scale of plant damage by rust has been developed. Based on results obtained, highly resistant (R 0%), resistant (R 11.2%) and weakly resistant (R 37.2–49.6%) cultivars were identified.

Keywords: aecium, *Astilbe chinensis*, collection, cultivar resistance, enphytoty, galls, *Pucciniostele mandschurica*, pustules, rust, spermogonia, spots, symptoms, telium

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INTRODUCTION

The rusts represents a dangerous infectious plant disease. The high adaptability of the pathogen to the host plant and the ability to produce a great number of spores during the growing season of the host plant contribute to its wide distribution. They affects both wild and cultivated plants. Currently, cultivars of the genus *Astilbe* are of special interest as valuable and very popular garden plants. The cultivars were repeatedly awarded the Royal Horticultural Society's Award of Garden Merit for decorative qualities and resistance to diseases and pests. The rust infection of astilbe cultivars has not been previously reported in the literature.

The rust fungus *Pucciniostele mandschurica* Deit. (*Phakopsoraceae*) is a demicyclic autoecious species parasitizing on *Astilbe chinensis* (Maxim.) Franch. et Sav. It was first found in Northeast China, Jilin Province, in 1896 by V.L. Komarov (Azbukina, 2005). Subsequently, the results of morphological and cytological studies were published (Kursanov et al., 1936). According to Z.M. Azbukina (1984, 2005, 2015), in Russia *P. mandschurica* is distributed in the southern part of the Far East (Amur Oblast, Khabarovsk Krai, and Primorsky Krai). Outside Russia, the fungus is found in northern and eastern China (Cummins, Ling, 1950; Liu, Tian, 2014; Weng et al., 2017), Korea, and Japan (Ono, 2017, 2019).

P. mandschurica was found on astilbe cultivars in the Botanical Garden-Institute, Far Eastern Branch, Russian Academy of Sciences (BGI FEB RAS) in 2012 (Shelekhova, 2013). In subsequent years, the spread of the disease was observed over the astilbe collection, so there was a need to study the features of rust damage to astilbe cultivars and evaluate resistance of cultivars to the disease.

The objectives of the study were as follows: to assess distribution of *P. mandschurica* in the collection, to describe the symptoms of the disease and characterize the general condition of the plants, to develop a scale to classify degrees of rust damage to leaves and flower stalks of astilbe cultivars, and to reveal rust-resistant astilbe cultivars.

MATERIAL AND METHODS

Astilbe (*Saxifragaceae*) is a perennial, herbaceous, rhizomatous plant with semi-rosette straight shoots. Leaves are bifoliate or trifoliate, rarely unifoliate. Flowers are small, numerous, gathered in the upper inflorescence panicles.

The astilbe collection at BGI FEB RAS consists of 75 cultivars from 10 garden groups and occupies an area of about 150 m^2. Cultivars are arranged on the basis of garden groups and decorative effect. We evaluated the condition of generative plants at 5–6 years of age.

The observations were carried out in the period from the beginning of the growing season to the completion of flowering against the natural infection background, i.e., without preliminary treatment of plants with fungicides. The 'Brautschleier', 'Amethyst', 'Weisse Gloria', 'Koknese', 'Kvele' cultivars, representing the full range of degrees of rust damage, were selected as objects of study. All plants in the collection were examined; prevalence of disease was visually evaluated and then calculated as the ratio of the number of affected to the number of examined plants, expressed in %. Symptoms of the disease were recorded: decrease in plant height; decrease in foliage density; deformation of leaves and inflorescences; number, size, and shape of pustules on leaves and flower stalks. The area of leaves and flower stalks affected by rust was scored on a scale of 0 to 4. The scale was designed by the authors of the present study for astilbe cultivars.

The scale to assess damage to leaves and flower stalks of *P. mandschurica*:

Score 0: leaves and flower stalks without visible spots and pustules; leaf color typical for the cultivar; no signs of damage.

Score 1: single small pustules covering no more than 5% of the surface of each leaf and flower stalk.

Score 2: few, scattered pustules, covering 10–20% of the surface of each leaf and flower stalk.

Score 3: numerous small- and medium-sized pustules, or single large pustules, powdering, covering 20–30% of the surface of each leaf and flower stalk.

Score 4: large pustules, covering from 30% to 60% or more of the surface area of each leaf and flower stalk.

For each cultivar, the intensity of damage from *P. mandschurica* (R, %) was calculated by the formula: $R = \frac{\sum n \cdot b}{N \cdot k} \cdot 100$ (Pospelov et al., 1986), where $\Sigma n \times b$ is the sum of the products of the number of affected leaves and flower stalks (n) by the respective score (b) on the 0–4 point scale; N is the total number of examined leaves and flower stalks; k is the highest score on the scale.

Freshly collected samples of affected plants were examined in the laboratory using an Axioplan 2 microscope (Carl Zeiss, Germany, 2011) with Plan-Neofluar 20×/0.5 and Plan-Neofluar 40×/0.75 lenses. Photographs were taken with an Axiocam ICc 3 digital camera mounted on a microscope and using the AxioVision 13 software. Images of the surface of affected parts were obtained on a Scan 7000 scanner (Pentacon, Germany, 2012).

The Latin names of fungi, plants, and abbreviations of the author's names are given in according to the open databases Index Fungorum (2018) and International Plant Names Index (2012).

RESULTS AND DISCUSSION

In the collection, *P. mandschurica* was first found on the 'Bronzelaub'*Astilbe* × *japonica* cultivar in 2012 (Shelekhova, 2013). By the sixth year of observations, the number of plants with different degrees of visible signs of the disease was 87 out of 158 samples of the collection, or 55% prevalence of disease. The decorative qualities of plants decreased significantly. Flower stalks and leafstalks were covered with swellings due to severe tissue hypertrophy, and leaves had pustules and spots (Fig. 1); flower stalks developed shorter and inflorescences were smaller. Over several years, the infection spread within a limited area of BGI where a large number of host plants were located. Therefore, currently the spread of *P. mandschurica*, the pathogen of astilbe rust, can be considered enphytoty, a local infectious disease.

In the process of observation, characteristic symptoms of astilbe rust were identified. The first rust spots appear on leaves in mid-May. In late June, numerous aecia with aeciospores and summer teliospores develop on leafstalks, leaf laminas, and flower stalks. The types of rust damage are leaf spotting, galls, swellings, pustules, and deformation of the axial organs of the plant and leaf laminas (Fig. 1). The spotting has the appearance of small yellow spots on the upper side of leaves due to the development of subcuticular spermagonia. The galls on the leafstalks and flower stalks are formed due to the hypertrophy of parenchyma, as a result of the development of aecia. In late June, the galls become large, up to 1.5 cm; some of them open and powder with orange spores, while others, having a purple color, remain closed (Fig. 1). Depending on the astilbe cultivar, galls can remain closed for a long time. Small pustules which remain closed for a protracted period are a sign of resistance.

Cultivars with green and red-brown leafstalks had different manifestations of damage. The infected plants of the susceptible cultivars had young, growing leaves that were deformed, twisted, and underdeveloped, which led to dwarfism and thinning of heavily damaged plants. Besides these signs, some of the cultivars formed a halo of necrotic tissue around large pustules which prevented nutrients from entering the pustule, rejecting it. Pustules of some cultivars were surrounded by a chlorotic halo of yellow or purple color, indicating resistance to rust, while susceptible astilbe cultivars had intensely powdering pustules surrounded by green tissue.

We studied fresh plant material without using fixatives and dyes, which allowed us to make some clarifications of the morphological structure of *P. mandschurica*. On the upper side of leaves, spermogonia type 7 (group VI) was observed, which corresponds to the classification (Hiratsuka, Sato, 1982). The size of spermogonia was 200–210 × 60–80 μm, which was slightly larger than those described in the literature (Azbukina, 2005). It was noted that spermatia were oval or oblong, having a size of 2–6 (8) × 2–3 μm (Fig. 2, A). Mature powdering aecium was 400 μm in height with aeciospores of 20–22 μm in diameter (Fig. 2, B).

In cross-sections of damaged leaves, the contours of parenchyma cells were visible due to partly preserved remnants of cell walls that had turned brown. The cell diameter was 50 μm; the cells were filled with starch.

Fig. 1. Lesions caused by *Pucciniostele mandschurica*: *a* – leaf spots; *b* – pustules; *c* – deformation of petioles and leaves; *d* – swelling; *e* – galls.

The colorless mycelium hyphae which filled the intercellular spaces were visible, with denser mycelium near stomata (Fig. 2, C).

In cross-section of a leafstalk taken through a purple-colored swelling 2–3 layers of parenchyma cells with an altered, anthocyanin color were located under the epidermis, with a primordium of aecium underneath. Around the aecium, photosynthetic cells with chloroplasts were partially preserved. The deeper layers of parenchyma were filled with starch grains (fig. 2, D).

There were telia with chains of teliospores 15–17 (20) μm × 27–29 μm in size visible on the lower side of the leaf; the maternal dividing cell of teliospore had four nuclei (Fig. 2, E). In the chains were teliospores with highly visible germinant pores. (Fig. 2, F).

The condition of astilbe cultivars damaged by rust fungus was evaluated in early July. The examined cultivars were in the phase of budding or onset of flowering. The maximum damage to plants from rust was usually observed during this period. The approach to evaluating the condition of astilbe cultivars in the collection was designed taking into account disease symptoms. To characterize them, we selected cultivars that differed in symptoms to the greatest extent (table 1). In further work, this approach can be applied to distinguish resistant cultivars and to determine protective measures. It should be noted that no very poor condition, let alone death, of plants from, rust fungus was observed in the collection. When severely damaged by rust infection, the plants still weakly bloomed. Besides rust, on the astilbe plants we observed the secondary saprotrophic fungi *Alternaria alternata* (Fr.) Keissl. and *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, which are pathogens of associated diseases. Damage caused by *Alternaria alternata* was manifested in the form of small brown speckles on young leaves. *Clado-*

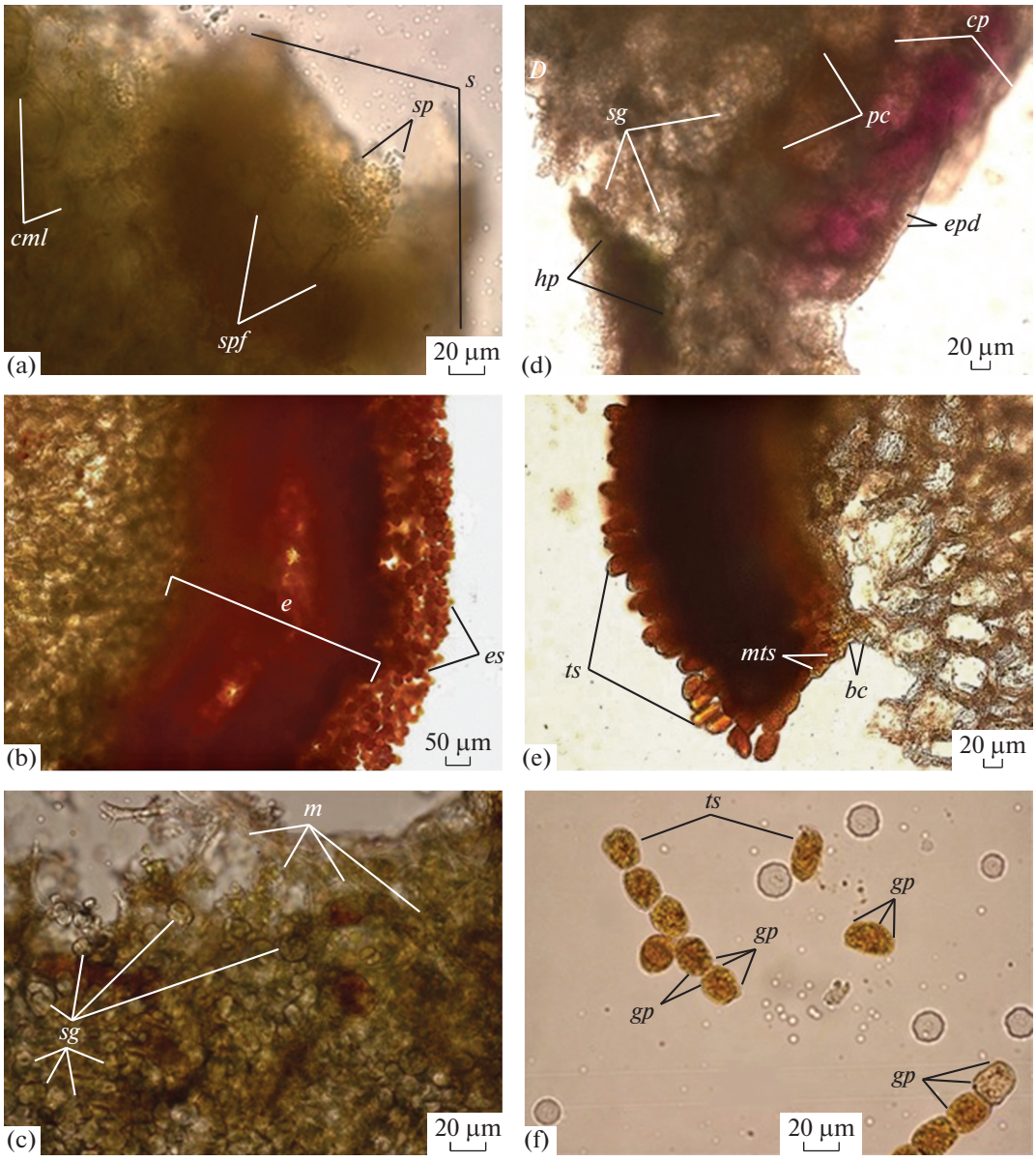

Fig. 2. Life stages of *Pucciniostele mandschurica*: a – spermogonium; b – aecium with aeciospores; c – section through affected leaf; d – primordium of aecium; e – telium; f – chains of teliospores; *bc* – basal cells; *cml* – leaf mesophyll cells; *cp* – parenchyma cells having an altered, purple color; *e* – aecium; *epd* – epidermis; *es* – aeciospores; *gp* – germ pores; *hp* – chloroplasts; *m* – mycelium; *mts* – maternal cells of the teliospore; *pc* – primordium of aecium caeomoid; *s* – spermogonium boundaries; *sg* – starch grains; *sp* – spermatia; *spf* – spermatophores; *ts* – teliospores.

sporium cladosporioides caused edges of sepals to turn brown.

To calculate the damage from rust (R, %), we determined the proportion (%) of the surface area of each leaf and flower stalk covered with pustules; then the result was scored (from 0 to 4) on the proposed original scale. In addition to differences in leaf area covered with pustules, sizes of pustules were taken into account: small (0.2–1 mm), medium (1–5 mm), or large (5–10 mm and more). Results of calculations of developmental intensity of the disease are presented in Table 2.

Analysis of the findings showed that the intensity of damage by rust differed between cultivars that were examined. The disease caused a decrease in plant density and plant height, and deformation of leaves and inflorescences. Pustules of various sizes and shapes developed on leaves and flower stalks (Tables 1–2).

The cultivar ‘Brautschleier’ plants did not have signs of damage from rust and the general condition was evaluated as excellent. According to the data of 2018, the plant height was below the mean long-term value (Table 2). This can be explained by the specific features of the growing period: differences in soil moisture, temperature deviations, amount of precipitation, and the individual aging processes. The ‘Brautschleier’

Table 1. State of astilbe cultivars in the collection of Botanical Garden-Institute, Vladivostok

State	Disease symptoms	Cultivar
Excellent	Plant density is even; height is uniform. Plants are robust, healthy, with well-developed numerous inflorescences	'Brautschleier'
Good	Plant density is insufficiently even. There are small differences in height and slight thinning of leaves between plants. Growth of the vegetative mass is somewhat slow, with a generally good condition of cultivar specimens. On some leaves there are small, barely visible rust pustules	'Amethyst', 'Weisse Gloria'
Medium	Density of standing plants is not completely even. Plant height and number of leaves are average; inflorescences are of medium size. Rust pustules on most leaves are single, small and medium in size. Plants have damage from associated diseases	'Koknese'
Bad	Plantings are thinned; density of standing plants is uneven. The number of leaves and height of inflorescences are significantly reduced. Some leaves and flower stalks are deformed; edges of leaves are twisted; parts of leaf free of pustules have a yellow color. Pustules on many leaves are open, powdering. Plants have an afflicted appearance	'Kvele'
Very bad	Inflorescences are weak, single, or flowering is absent. Complete or almost complete death of plants	–

Table 2. Damage intensity of examined cultivars by *Pucciniostele mandschurica* (2018)

Cultivar	N	Damage, score					$\Sigma n \times b$	R, %	Plant height, cm	
		0	1	2	3	4			2018	Mean long-term
		Number of leaves and flower stalks corresponding to the score								
'Brautschleier'	107	107	–	–	–	–	0	0	35.7	39.4
'Amethyst'	158	87	71	–	–	–	71	11.2	80.5	100.5
'Weisse Gloria'	58	45	8	1	–	4	26	11.2	20.4	29.5
'Koknese'	41	11	14	8	1	7	61	37.2	57.6	84.5
'Kvele'	57	17	7	9	8	16	113	49.6	28.5	44.2

cultivar was characterized as highly resistant to the disease.

Insignificant intensity of damage from rust (R 11.2%) was recorded for the 'Amethyst' and 'Weisse Gloria' cultivars. The proportion of leaves and flower stalks without signs of disease was 55 and 77%, respectively. Decrease in plant height can be explained by specifics of the growing season. The general condition of the plants was good (Tables 1–2). Both cultivars were characterized as rust-resistant.

The 'Koknese' (R 37.2%) and 'Kvele' (R 49.6%) cultivars were affected by rust to the greatest degree. The proportion of leaves and flower stalks with signs of the disease was more than 70%; the plant height decreased by almost 35% (Table 2). Development of the disease was reflected in the general condition of the plants, however, without causing death. In regard to rust, cultivars 'Koknese' and 'Kvele' were characterized as weakly resistant.

Thus, in the collection of BGI FEB RAS, spread of *Pucciniostele mandschurica* and the development of disease of astilbe cultivars are characterized as enphytoty: a rapid and mass infection of plants within a limited area. More than 55% of cultivars have signs of damage from rust: leaf spotting, galls, swellings, pustules, deformation of flower stalks, infloresences and leaf laminas, and decreased height of the plant. Based on manifestation of signs of disease, the condition of different astilbe cultivars is characterized as "excellent," "good," "medium," or "bad." At the present time, there are no plants in "very bad" state and no case of plant death due to rust infection. Along with rust disease signs appear of infection by saprotrophic fungi such as *Alternaria alternata* and *Cladosporium cladosporioides*, which are pathogens of associated diseases. The scale of astilbe rust damage designed by the authors of this article makes it possible, using the corresponding grade, to estimate the percentage (%) of damaged area of each leaf and flower stalk. Calculation of the intensity of damage (R 0%) of the *Pucciniostele mandschurica* cultivars that were examined and analysis of findings showed that the 'Brautschleier' cultivar is characterized as highly resistant (R 0%). The 'Amethyst' and 'Weisse Gloria' cultivars are resistant (R 11.2%)

to the disease. The 'Koknese' and 'Kvele' cultivars are weakly resistant (R 37.2% and R 49.6%, respectively) to rust infection.

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Pucciniostele mandschurica – возбудитель ржавчины сортовых астильбе

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В Ботаническом саде-институте ДВО РАН на сортовых астильбе обнаружен ржавчинный гриб *Pucciniostele mandschurica*. Распространение возбудителя ржавчины в коллекции характеризуется как энфитотия. Описаны симптомы инфицирования растений *P. mandschurica* (снижение высоты растений и плотности листвы, деформация соцветий, пустулы, галлы, желтые пятна на листьях и цветоносах). С учетом проявления симптомов заболевания дана оценка состояния растений астильбе в коллекции (отличное, хорошее, среднее, плохое). Для расчета интенсивности развития заболевания (R,%) разработана оригинальная пятибалльная (от 0 до 4) шкала учета поражения растений ржавчиной. На основании полученных результатов выявлены высоко устойчивые сорта (R 0), устойчивые (R 11.2) и слабоустойчивые (R 37.2–49.6).

Ключевые слова: галлы, коллекция, пустулы, пятна, ржавчина, симптомы, сорт, спермогоний, телий, устойчивость сортов, энфитотия, эций, *Astilbe chinensis*, *Pucciniostele mandschurica*