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THE MAIN WAYS OF INFECTION OF GRAIN SORGHUM BY *BURKHOLDERIA ANDROPOGONIS* (*BURKHOLDERIACEAE*, *BURKHOLDERIALES*) IN THE FOREST-STEPPE OF THE MIDDLE VOLGA REGION

ОСНОВНЫЕ ПУТИ ЗАРАЖЕНИЯ ЗЕРНОВОГО СОРГО *BURKHOLDERIA ANDROPOGONIS* (*BURKHOLDERIACEAE*, *BURKHOLDERIALES*) В ЛЕСОСТЕПИ СРЕДНЕГО ПОВОЛЖЬЯ

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Bacterial leaf stripe, caused by Burkholderia andropogonis, is one of the main sorghum diseases in the forest-steppe of the Middle Volga region. The purpose of the research was to examine the main ways of infection of sorghum by Burkholderia andropogonis. The studies were carried out on experimental fields of the Volga region Scientific Research Institute of Selection and Seed-growing and in the laboratories of Plant Protection and Microbiology of the Samara State Agricultural Academy in 2010–2015. The severity of sorghum leaf stripe in the stage of physiological maturity was 9–60%, with a maximum in high-drought (2010), minimum in wet and cool (2011) years. Losses of grain sorghum yield from B. andropogonis were on average up to 30 %. The main overwintering and primary inoculum sources reported are infested seeds. Up to 97–100 % of the sorghum seeds were infected with B. andropogonis. After sowing infected seeds, bacteria spread through the vascular system in seedlings and developing plants, penetrating into emerging and ripening seeds. The main indirect vector of bacterial leaf stripe is the flea beetle Phyllotreta vittula feeding on sorghum leaves. In the first half of July, adults of the new generation of P. vittula facilitate the penetration of the pathogen from B. andropogonis exudate into the tissues of sorghum through wounds that feeding beetles form on the upper surface of young leaves. The role of flea beetles P. vittula in the spread of the bacteria B andropogonis in crops of sorghum recorded for the first time.

Key words: grain sorghum, seeds, bacterial leaf stripe, exudate, leef-beetles, Phyllotreta vittula.

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Бактериальная полосатость листьев, вызванная патогеном Burkholderia andropogonis, одно из основных заболеваний зернового сорго в лесостепи Среднего Поволжья. Цель исследования заключалась в изучении основных путей заражения сорго бактерией B. andropogonis. Исследования проводились на опытных полях Поволжского НИИ селекции и семеноводства и в лабораториях защиты растений и микробиологии Самарской государственной сельскохозяйственной академии в 2010–2015 гг. Интенсивность развития бактериальной полосатости листьев в стадии физиологической спелости зерна сорго составила 9–60%, с максимумом в засушливые (2010), минимумом во влажные и прохладные (2011) годы. Потери урожая зернового сорго от B. andropogonis составили в среднем до 30 %. Основным источником заражения зернового сорго этим патогеном являются инфицированные семена, количество которых составляло до 97–100 %. После посева инфицированных семян бактерии распространяются по проводящей системе в проростках и развивающихся растениях, проникая в формирующиеся и созревающие семена, где проходит их зимовка. Другой важный путь распространения патогена – экссудат, содержащий бактерий и выделяющийся на нижней стороне листьев сорго. Благоприятную среду для развития и проникновения разносимых ветром, дождем, насекомыми бактерий из экссудата в ткани листьев сорго создают свежие погрызы на верхней стороне молодых листьев сорго имаго полосатой блошки Phyllotreta vittula нового поколения, что наблюдалось в первой половине июля. Роль P. vittula в распространении бактерий B. andropogonis в посевах сорго отмечена впервые.

Ключевые слова: зерновое сорго, семена, экссудат, бактериальная полосатость листьев, жуки-листоеды, Phyllotreta vittula.

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Sorghum is the 5th most important cereal grain crop in the world after wheat, rice, corn and barley. It is grown on the area about 50–60 million ha (Delserone, 2007). In Russia, sorghum is grown in a small area (approximately 65 thousand ha) with a low yield (0.8–1.7 t/ha). One of the reason for the low yield of sorghum in the Volga region is bacterial diseases.

The economically important bacterial diseases of sorghum are bacterial leaf spot (*Pseudomonas syringae* van Hall, 1904 (=*Pseudomonas holci* Kendrick), bacterial leaf streak (*Xanthomonas holcicola* (Elliot, 1930) and bacterial leaf stripe (*Burkholderia andropogonis* (Smith, 1911) Gillis *et al.*, 1995 (=*Pseudomonas andropogonis* (Smith) Stapp) (Li, 1993; Gillis *et al.*, 1995; Frederickson & Odvody, 2000; Navi *et al.*, 2002; Ramundo & Claflin, 2005; Georgieva-Andreeva *et al.*, 2011).

Bacterial leaf stripe affects more than 50 species of plants belonging to 15 families, prefers cereals and legumes. The most important sources of infection are infected seeds, residues on the surface and in the upper layer of the soil, jonssonova grass (*Sorghum halepense L.*) and other perennial weeds. The pathogen can remain viable up to three years or more in seeds stored under optimum conditions in the laboratory. The pathogen spreads from plant to plant by wind, wind-driven rain, cultivation when foliage is wet, but mainly through infected seeds. Infection enters plants

through wounds or natural openings (stomata). Bacterial leaf diseases are controlled by using clean seeds, crop rotation, and clean cultivation to destroy plant residues (Gorlenko, 1966; Pastushenko & Belevich, 1971; Chumaevskaya, 1977; Edmunds & Zummo, 1975; Li, 1993; Hseu *et al*., 2007; Krawczyk *et al*., 2010; Almeida *et al*., 2009).

The symptoms of the disease are well marked in the milk and dough sorghum growth stages. They well differ in form and color spots, the presence and color of bacterial exudate on the undersurface of leaves (Leukel *et al.,* 1951). Striped spots light brown, red, dark purple to almost black, merging lanes without a border are formed on leaves, sheaths and stems. Exudate is released on the leaf surface through the stomata. This exudate dries to a thin, red or redish scales (Pastushenko & Belevich, 1971). The systematic position of the pathogen causing bacterial leaf stripe of sorghum is not very clear. Many researchers attribute it to the genus *Burkholderia* Yabuuchi & al., 1993, including more than 60 species (Yabuuchi *et al*., 1993; Palleroni, 2005; Ramundo & Claflin, 2005; Santos *et al*., 2013).

In Russia bacterial leaf stripe of sorghum was identified in 1973 (Yakushevsky *et al*., 1974). However, resistance of sorghum cultivars to this disease has not been studied.

Barley flea beetle (*Phyllotreta vittula* (Redtenbacher, 1849)) has one generation per year with overwintering adults in the soil, leaf litter. The beetles emerge from hibernation sites early in the spring and feed on leaves of cruciferous weeds, autumn seedlings of wheat and barley and other cereals. Later they migrate to the seedling of spring wheat, barley for further feeding. Host plants of *P. vittula* belong to several families, first of all Poaceae and Brassicaceae and also Chenopodiaceae, Amaranthaceae, Cannabiaceae, Polygonaceae, Asteraceae, Linaceae, Tropaeolaceae (Vig, 1998). Larvae of *P. vittula* develop in the soil. Adults gnaw small pits or holes on the upper epidermis and parenchyma of the leaves. Overwintering adults are active to the end of May-beginning of June. Adults of the new generation occure from the end of June–beginning of July to the second part of October–beginning of November. Flee beetle larvae and adults cause indirect damage by transmission of plant pathogens from infected host plants to healthy during feeding, such as *Alternaria brassicicola* to cabbage, *Xanthomonas campestris*, Yellow mosaic virus, Brome mosaic virus (Toshova *et al.,* 2009).

The purpose of the research was to examine the main ways of infection of grain sorghum (*Sorghum bicolor* (L.) Moench.) (cultivar Premiera) by the pathogen *Burkholderia andropogonis*.

Materials and methods

The studies were carried out on experimental fields of the Volga region Scientific Research Institute of Selection and Seed-growing named after P.N. Konstantinov and Samara State Agricultural Academy in 2010–2015. The soil is ordinary chernozem, clay loamy.

Sorghum plants naturally infected with bacterial leaf stripe were observed in the field in the sorghum growth stages of emergence, booting, flowering, milk, soft dough and physiological maturity (Shurovenkov, 1984). Identification of the affected plants and the pathogen was carried out in the field by external manifestations of the disease on sorghum leaves, the presence and color of exudate and in the laboratory of microbiology of the Samara State Agricultural Academy with using of PCR analysis (Bagsic *et al*., 1995). The infected leaf tissues, stems and roots of laboratory seedlings were used for identification of *B. andropogonis*.

Fifty plants of sorghum with three replications were examined on plots during measurements of bacterial leaf stripe. The main elements of disease measurement were Incidence (I) (the number or proportion of diseased plants in a population) and Severity (S) (the area or proportion of plant tissue that is symptomatic) (Kosov & Polyakov, 1958).

The infestation of sorghum seeds with a leaf stripe was determined by a laboratory test for germination. Superficial infection on the seeds was removed with running water on a sieve for 2–3 minutes, than seeds were placed for 20–30 sec in 95% ethanol and transferred to sterile Petri dishes on damp sterile filter paper for 25 seeds in three replications. Sterile water were used to moisten the seeds in Petri dishes. Closed dishes with seeds were placed in a thermostat, where they were kept for 7 days at a temperature of 28 °C. Laboratory seed germination was determined after 7 days, the color of embryonic organs was also noted and microscopic preparations of non-viable seeds and embryonic organs (root, steam and leaf) of seedlings were made by the traditional methods of microbiological studies. The preparations were stained with a solution of fuchsin and viewed under the microscope using the lens x100 and oil immersion. It was found that seedlings infected by *B. andropogonis* had a reddish-pink color.

Beetles of *Phyllotreta vittula* (Redtenbacher, 1849) and their damages to sorghum leaves were taken into account visually by examining 50 sorghum plants in three replications in the morning and evening hours of July–August once in 5 days.

Results and discussion

Microscopic analysis of germinated seeds of grain sorghum in laboratory conditions. It was found that embryonic organs (leaves, steams and roots) of sorghum seedlings affected by the bacteria *Burkholderia andropogonis*, had a reddish-pink color. Laboratory analysis of the seeds of the 2010–2012 harvest, conducted in April 2015, showed a high viability of bacteria. 97–100% of the sorghum seedlings were infected with bacteria (Table 1).

Low laboratory germination of the sorghum seeds in 2011 was due to high humidity in the ripening stage of sorghum. 198.5 mm of rain fell in September. Molding of seeds was observed in field conditions. *Fusarium* and *Alternaria* were developing on seeds.

Development of Burkholderia andropogonis in the field. In the field in the emergence stage of sorghum, the bacteria *B. andropogonis* begin to spread into the vascular system of plants. The reddish-pink coloration appears primarily on the stems and along the veins in the leaves of sorghum affected with bacteria. The first symptoms of the diseases were observed in the flag leaf stage of sorghum. At

Таблица 1.

Laboratory germination and infection with Burkholderia andropogonis of the grain sorghum seeds, cultivar Premiere (data analysis seeds in April 2015)

Year	Laboratory germination, %	Seedlings infected with bacteria, %			Healthy seedlings, %
		shoot and root	root	total	
2010	83.0 ± 5.1	64.7 ± 3.1	35.3 ± 1.5	100	0
2011	43.0 ± 2.5	61.1 ± 2.6	35.4 ± 1.2	96.7	3.3
2012	90.2 ± 4.9	59.2 ± 2.8	38.8 ± 1.6	98.0	2.0

this stage, a reddish-pink exudate containing bacteria can be found on the undersurface of the leaves. Thin reddish scales are formed as the exudate dries. Bacteria from the exudate spread to other plants of sorghum by the wind, rain, insects. Through the stomata they penetrate into the parenchyma of leaves, where their development in the intercellular space leads to the formation of small, then increasing in size, elongated spots, merging with each other and covering a significant part of the sheet. Bacteria penetrate into the forming grain by the vascular system in the stages of flowering, grain formation and milk ripeness, where obtain their nutrition, complete development, and overwinter in the seeds. Sowing of bacteria-infected seeds causes infection of new generation plants. The severity of the leaf stripe in the stage of physiological maturity of grain sorghum was 60 ± 8, 9 ± 2, 46 ± 5 and 26 ± 3 %, respectively in 2010, 2011, 2012 and 2013, with a maximum in high-drought (2010), minimum in wet and cool (2011) years. Losses of grain sorghum yield from *B. andropogonis* were on average up to 30 %.

It was established that bacteria *B. andropogonis* may infect the leaves of other plants through mechanical damages caused mainly by insects with chewing mouthparts. Among them in the forest-steppe of the Middle Volga region the main indirect vector of bacterial leaf stripe is the flea beetle *Phyllotreta vittula* feeding on sorghum leaves. *P. vittula* has one generation per year with overwintering of adult beetles in the soil and leaf litter. In spring the beetles concentrate in the fields of spring wheat and barley, where they feed on the leaves of seedlings. Females lay eggs in the top layer of the soil around the base of the host plants, where the larvae develop and pupate. Beetles of new generation appear in the first half of July in the boot stage of sorghum and feed on the upper surface of young sorghum leaves. *P. vittula* adults of the new generation indirect facilitate the penetration of pathogenic bacteria with their exudate into the host plants through the wounds that feeding beetles form on the leaves. A typical external symptom of leaf stripe in the form of reddish coloring around the mechanical damage of the flea beetles was observed five-ten days after the entry of bacteria into leaf tissue within these lesions. In the first decade of July 2015, the number of flea beetles in sorghum crops was 5–10 specimens per plant. Microscopic analysis of infected tissue in the laboratory showed the presence of bacteria *B. andropogonis*. The role of flea beetles *Phyllotreta vittula* in the spread of the bacteria *B andropogonis* in crops of sorghum recorded for the first time.

Conclusion. Leaf stripe, caused by the bacterial pathogen *Burkholderia andropogonis*, is a serious threat to sorghum production in the forest-steppe of the Middle Volga region. The severity of leaf stripe in the stage of physiological maturity was 9–60%, with a maximum in high-drought (2010), minimum in wet and cool (2011) years. Losses of grain sorghum yield from *B. andropogonis* were on average up to 30 %. The main overwintering and primary inoculum sources reported are infested seeds. Up to 97–100 % of the sorghum seeds were infected with *B. andropogonis.* After sowing infected seeds, bacteria spread through the vascular system in seedlings and developing plants, penetrating into emerging and ripening seeds. The main indirect vector of bacterial leaf stripe is the flea beetle *Phyllotreta vittula* feeding on sorghum leaves. In the first half of July, adults of the new generation of *P. vittula* facilitate the penetration of the pathogen from *B. andropogonis* exudate into the tissues of sorghum through wounds that feeding beetles form on the upper surface of young leaves. The role of flea beetles *Phyllotreta vittula* in the spread of the bacteria *B andropogonis* in crops of sorghum recorded for the first time.

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